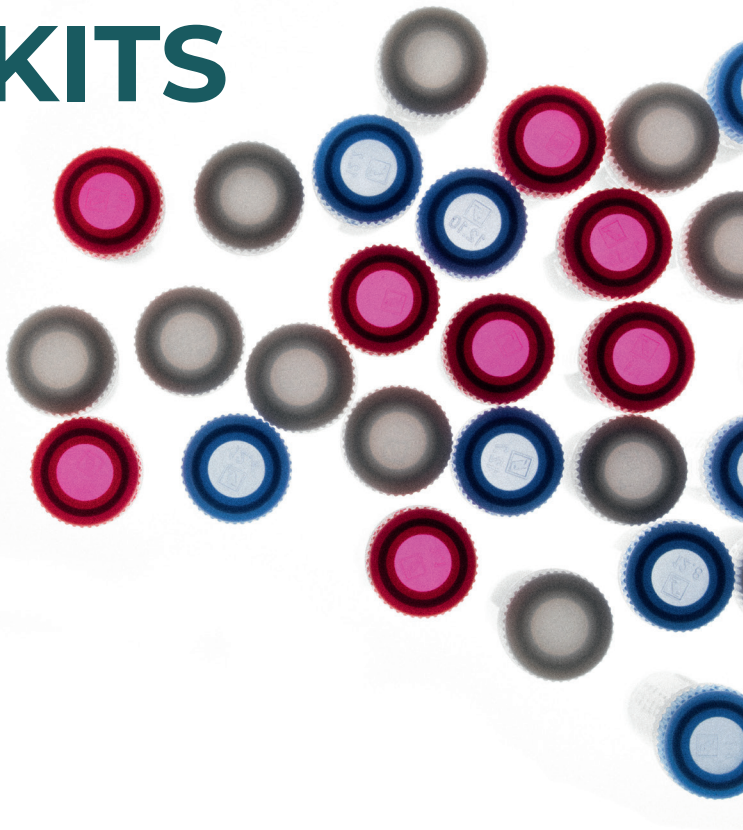




**SSI**  
DIAGNOSTICA

Instructions For Use

# PCR KITS



ENGLISH



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# GENERAL INFORMATION

## Quality Certificate

SSI Diagnostica is quality assured and certified in accordance with ISO 13485. Certificate of analysis can be downloaded from our website [www.ssidiagnostica.com](http://www.ssidiagnostica.com).

## Support

Additional information about the PCR Kits is available at our homepage [www.ssidiagnostica.com](http://www.ssidiagnostica.com).

If you have any difficulties using the products, please contact SSI Diagnostica at [info@ssidiagnostica.com](mailto:info@ssidiagnostica.com).

## ABBREVIATION LIST

|                                           |                                                       |
|-------------------------------------------|-------------------------------------------------------|
| A/EEC                                     | Attaching and effacing <i>E. coli</i>                 |
| <i>A. flavus</i>                          | <i>Aspergillus flavus</i>                             |
| <i>A. fumigatus</i>                       | <i>Aspergillus fumigatus</i>                          |
| <i>A. niger</i>                           | <i>Aspergillus niger</i>                              |
| <i>A. wentii</i>                          | <i>Aspergillus wentii</i>                             |
| <i>C. albicans</i>                        | <i>Candida albicans</i>                               |
| <i>C. glabrata</i>                        | <i>Candida glabrata</i>                               |
| <i>Chs1</i>                               | Chitin synthase 1                                     |
| <i>C. krusei</i>                          | <i>Candida krusei</i>                                 |
| <i>C. parapsilosis</i>                    | <i>Candida parapsilosis</i>                           |
| <i>C. tropicalis</i>                      | <i>Candida tropicalis</i>                             |
| DEC                                       | Diarrhoeagenic <i>E. coli</i>                         |
| EAEC                                      | Enteraggregative <i>E. coli</i>                       |
| <i>E. coli</i>                            | <i>Escherichia coli</i>                               |
| <i>E. floccosum</i> var. <i>Floccosum</i> | <i>Epidermophyton floccosum</i> var. <i>Floccosum</i> |
| EHEC                                      | Enterohaemorrhagic <i>E. coli</i>                     |
| EIEC                                      | Enteroinvasive <i>E. coli</i>                         |
| EPEC                                      | Enteropathogenic <i>E. coli</i>                       |
| ETEC                                      | Enterotoxigenic <i>E. coli</i>                        |
| <i>F. oxysporum</i>                       | <i>Fusarium oxysporum</i>                             |
| <i>F. proliferatum</i>                    | <i>Fusarium proliferatum</i>                          |
| <i>F. solani</i>                          | <i>Fusarium solani</i>                                |
| <i>Its2</i>                               | Internal transcribed spacer 2                         |
| LoD                                       | Limit of Detection                                    |
| <i>M. audouinii</i>                       | <i>Microsporum audouinii</i>                          |
| <i>M. canis</i>                           | <i>Microsporum canis</i>                              |
| <i>M. gypseum</i>                         | <i>Microsporum gypseum</i>                            |
| NTC                                       | Non-template control                                  |
| <i>S. brevicaulis</i>                     | <i>Scopulariopsis brevicaulis</i>                     |
| TE-Buffer                                 | Tris-EDTA Buffer                                      |
| <i>T. erinacei</i>                        | <i>Trichophyton erinacei</i>                          |
| <i>T. interdigitale</i>                   | <i>Trichophyton interdigitale</i>                     |
| <i>T. mentagrophytes</i>                  | <i>Trichophyton mentagrophytes</i>                    |
| <i>T. rubrum</i>                          | <i>Trichophyton rubrum</i>                            |
| <i>T. schoeuleinii</i>                    | <i>Trichophyton schoeuleinii</i>                      |
| <i>T. soudanense</i>                      | <i>Trichophyton soudanense</i>                        |
| <i>T. tonsurans</i>                       | <i>Trichophyton tonsurans</i>                         |
| <i>T. verrucosum</i>                      | <i>Trichophyton verrucosum</i>                        |
| <i>T. violaceum</i>                       | <i>Trichophyton violaceum</i>                         |

# DERMATOPHYTE PCR

## DERMATOPHYTE REAL TIME PCR KIT

### Intended use

The Dermatophyte Real Time PCR Kit is for *in vitro* diagnostic detection of dermatophytes in general (pan-dermatophytes) and specifically *T. rubrum* in nail specimens.

### Description

The kit contains the following PCR reagents: Primer and Probe Mix (includes three primer and probe sets and Internal Plasmid Control), *T. rubrum* Positive DNA Control and RT Supermix. Furthermore, it contains Buffer A and Buffer B for template preparation. Each of the primer and probe set for pan-dermatophytes and *T. rubrum* detection is designed for a specific region of the *its2* gene, as shown in table 1. The pan-dermatophyte amplification is detected by the fluorescent dye HEX and *T. rubrum* amplification is detected by the fluorescent dye FAM. The Internal Plasmid Control is detected by the fluorescent dye Cy5/Quasar 670. The *T. rubrum* Positive DNA Control contains genomic DNA from *T. rubrum* and serves both as control for the pan-dermatophytes and the *T. rubrum* amplification.

Table 1. Target detection of the Primer and Probe Mix.

| Target detection               | Gene               |
|--------------------------------|--------------------|
| Pan-dermatophytes <sup>a</sup> | <i>its2</i>        |
| <i>T. rubrum</i>               | <i>its2</i>        |
| Internal Plasmid Control       | Synthetic fragment |

<sup>a</sup> Dermatophytes in general e.g. *T. rubrum*, *T. mentagrophytes*, *T. interdigitale*, *T. tonsurans*, *T. schoeuleinii*, *T. violaceum*, *T. soudanense*, *T. verrucosum*, *T. erinacei*, *M. canis*, *M. audouinii*, *E. floccosum* var. *Floccosum*.

### Principle

Nail infections are mainly caused by *T. rubrum* and *T. mentagrophytes*. The traditional identification method with culturing and microscopic examination is time-consuming and varies from 10 days to 4 weeks<sup>1</sup>. This multiplex Real Time PCR based method detects dermatophytes in general, and specifically *T. rubrum* within 2-3 hours.

### Materials Provided

The reagents supplied in the kit are listed in table 2. The kit comprises reagents enough to perform 100 multiplex Real Time PCR reactions. The control vial contains 150 µL corresponding to at least 50 PCR tests.

Table 2. Reagents provided.

| Reagent                                 | Cap color | Volume |
|-----------------------------------------|-----------|--------|
| Primer and Probe Mix <sup>a</sup>       | Green     | 950 µL |
| <i>T. rubrum</i> (Positive DNA Control) | White     | 150 µL |
| RT Supermix <sup>b</sup>                | Red       | 1,1 mL |
| Buffer A                                | -         | 20 mL  |
| Buffer B                                | -         | 20 mL  |

<sup>a</sup> Primer and Probe Mix for detection of pan-dermatophytes, *T. rubrum* and Internal Plasmid Control. The plasmid used as template for amplification of the internal control is included in the mix.

<sup>b</sup> Enzyme master mix for probe-based Real Time PCR.

### Materials and Instruments Required but not Provided

- Tubes for template preparation
- Heat block or water bath (95°C)
- Vortex mixer
- Real Time Thermal Cycler, which contains FAM, HEX and Cy5/Quasar 670 channels (see table 3 for wavelengths)
- Computer connected to the Real Time Thermal Cycler for analysis of the results

The Dermatophyte Real Time PCR Kit has been validated on the following instruments: Bio-Rad CFX96™, Qiagen Rotor-Gene® Q, ABI 7500 and Roche LightCycler® 480.

### Precautions

The Dermatophyte Real Time PCR Kit has been developed for use with template DNA from patient nail specimens prepared using Buffer A and Buffer B (DNA preparation as described on page 8). If the kit is used with DNA template from cultured fungi, the DNA template must be diluted 100 times with Buffer A/Buffer B (ratio 1:1) or RNase/DNase free water before use.

## Procedure

Template preparation and PCR setup should be performed in dedicated areas free of possible contamination.

### DNA Preparation

1. Add 100 µL of Buffer A to the nail specimen. Incubate the sample at 95°C for 10 minutes\*.
2. Immediately add 100 µL of Buffer B and vortex. The sample is ready for PCR.

\*If the nail specimen is large, either cut the nail into small pieces or increase the volume of Buffer A to cover the sample. Increase the volume of Buffer B equally (see item 2).

### PCR Set-up

3. Prepare the master mix as described in table 4 for the number of samples to be run.
4. Dispense 18 µL of the master mix and 2 µL of template DNA (sample or positive control) in each tube. Prepare a negative control (NTC) by mixing 18 µL of the master mix and 2 µL Buffer A/Buffer B (ratio 1:1).
5. Set up the PCR protocol on a Real Time Thermal Cycler as described in table 5.
6. Select the fluorescence channel FAM (*T. rubrum*), HEX (pan-dermatophyte) and Cy5/Quasar 670 (Internal Plasmid Control) as described in table 3.
7. Run the PCR amplification in the Real Time Thermal Cycler.

Table 3. PCR target, dyes and detection wavelength (nm).

| Target                   | Dye            | nm      |
|--------------------------|----------------|---------|
| <i>T. rubrum</i>         | FAM            | 520     |
| Pan-dermatophytes        | HEX            | 556     |
| Internal Plasmid Control | Cy5/Quasar 670 | 669/670 |



Table 4. Preparation of master mix.

| Reagent              | Volume/reaction |
|----------------------|-----------------|
| Primer and Probe Mix | 8 µL            |
| RT Supermix          | 10 µL           |
| Total volume         | 18 µL           |

Table 5. Amplification protocol.

| Step                | Temp. (°C) | Time    | Cycle(s) |
|---------------------|------------|---------|----------|
| Pre-denaturation    | 95°C       | 2 min.  | 1        |
| Denaturation        | 95°C       | 10 sec. | } 40     |
| Annealing/extension | 64°C       | 1 min.  |          |

### Interpretation of the Analysis Results

In figure 1, the result of a positive *T. rubrum* sample is shown by three amplification curves.

Amplification curve detected by FAM (shown as a **blue** curve): The nail specimen is positive for *T. rubrum* DNA and the patient has a nail infection caused by *T. rubrum*.

Amplification curve detected by HEX (shown as a **green** curve): The nail specimen is positive for pan-dermatophytes and the patient has a nail infection caused by a dermatophyte. In this case the infection is determined to be *T. rubrum*.

Amplification curve detected by Cy5/Quasar 670 (shown as a **purple** curve): Detection of the Internal Plasmid Control. This signal should be observed in all tests, both positive and negative.

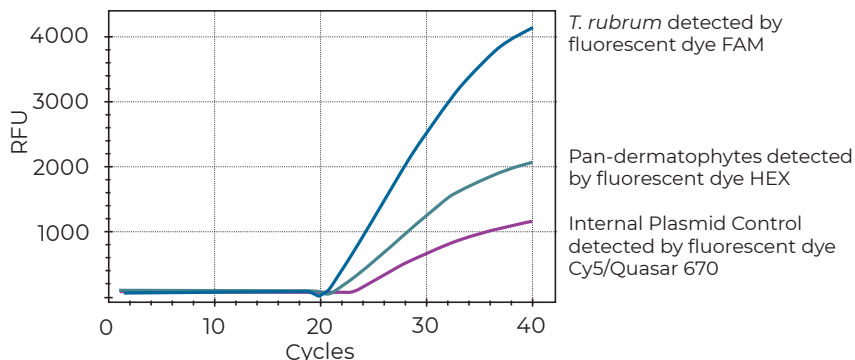


Figure 1: A *T. rubrum* positive nail specimen showing amplification curves for *T. rubrum* (FAM, **blue**), pan-dermatophytes (HEX, **green**) and Internal Plasmid Control (Cy5/Quasar 670, **purple**).

In figure 2 a pan-dermatophyte positive result is shown by two amplification curves.

Amplification curve detected by HEX (shown as a **green** curve): The nail specimen is positive for pan-dermatophytes and the patient has a nail infection caused by a dermatophyte. In this case the nail infection is not caused by *T. rubrum* since an amplification curve detected by FAM (**blue** curve) is not observed.

Amplification curve detected by Cy5/Quasar 670 (shown as a **purple** curve): Detection of the Internal Plasmid Control. This signal should be observed in all tests, both positive and negative.

In figure 3 the result of a nail sample not infected by a dermatophyte is shown by one amplification curve.

Amplification curve detected by Cy5/Quasar 670 (shown as a **purple** curve): Detection of the Internal Plasmid Control. This signal should be observed in all tests, both positive and negative.

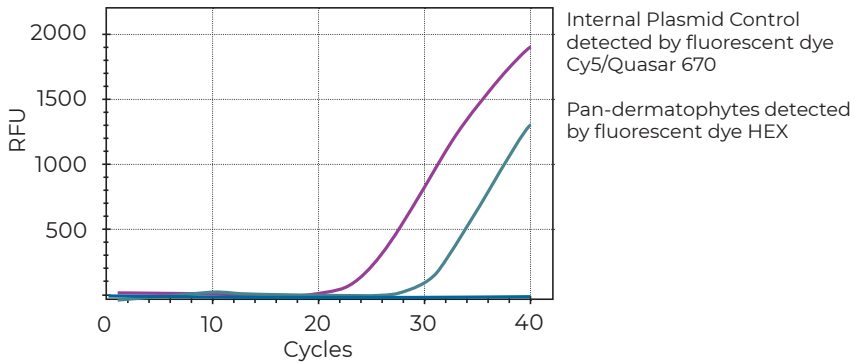


Figure 2: A pan-dermatophyte positive nail specimen showing amplification curves for, pan-dermatophytes (HEX, **green**) and Internal Plasmid Control (Cy5/Quasar 670, **purple**).

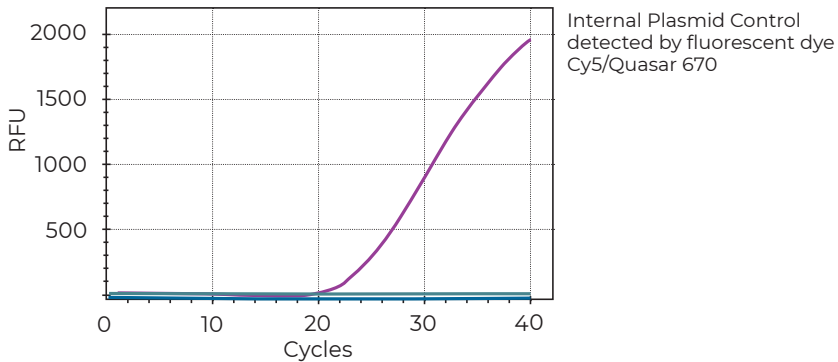


Figure 3: A negative nail specimen showing an amplification curve for the Internal Plasmid Control (Cy5/Quasar 670, **purple**).

The amplification curves should start increasing before the Ct cutoff values listed in table 6, to be a true positive result.

Table 6. Ct cutoff values for nail specimens and culture samples.

| Interpretation         | Channel<br>FAM<br>Blue | Channel<br>HEX<br>Green | Channel<br>Cy5/Quasar 670<br>Purple |
|------------------------|------------------------|-------------------------|-------------------------------------|
| <i>T. rubrum</i> pos.  | Ct < 37                | Ct < 35                 | Ct < 37                             |
| Pan-dermatophytes pos. | -                      | Ct < 35                 | Ct < 37                             |
| Negative               | -                      | -                       | Ct < 37                             |

## Specificity

The Dermatophyte Real Time PCR Kit showed 100% specificity for both *T. rubrum* and pan-dermatophyte detection, when testing a panel of DNA samples purified from cultures of *T. rubrum* (n=4) and other dermatophytes (*E. floccosum*, *M. canis*, *M. audouinii*, *M. gypseum*, *T. erinaceid*, *T. interdigitale*, *T. mentagrophyte*, *T. schoeuleinii*, *T. soudanense*, *T. tonsurans*, *T. verrucosum*, *T. violaceum*) (n=36). Also tested were 26 non-dermatophytes (*A. flavus*, *A. fumigatus*, *A. niger*, *A. wentii*, *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *Mallassezia furfur*, *S. brevicaulis*), where only amplification of the Internal Plasmid Control were observed, as expected.

## Sensitivity (LoD)

The Dermatophyte Real Time PCR Kit can detect less than 10 copies of genomic DNA of *T. rubrum* and pan-dermatophytes per reaction.

## Comparison Study

The Dermatophyte Real Time PCR Kit has been validated with a panel of 187 pan-dermatophyte positive (n= 82) and negative (n= 105) human nail specimens from a hospital. Compared to results obtained by the hospitals own in-house Real Time PCR test the kit showed 98% agreement.

## **Storage and Shelf Life**

The Dermatophyte Real Time PCR Kit is shipped with cooling elements but should be stored in a dark place at -20°C immediately on receipt. Avoid repeated thawing/freezing and direct light exposure of the Primer and Probe Mix, as this might reduce the stability of the reagent and thus affect the efficiency of the DNA amplification. If you plan to freeze/thaw the Primer and Probe Mix more than 10 times, aliquot out in tubes. The expiry date of the kit is printed on the label.

# DERMATOPHYTE CONVENTIONAL PCR KIT

## Intended use

The Dermatophyte Conventional PCR Kit is for *in vitro* diagnostic detection of dermatophytes in general (pan-dermatophytes) and specifically *T. rubrum* in nail specimens.

## Description

The kit contains the PCR reagents: Primer Mix, two PCR positive DNA controls (Control 1/pan-derm and Control 2/*T. rubrum*) and a PCR ReadyMix (including loading buffer). Furthermore, it contains Buffer A and Buffer B for template preparation. The Primer Mix contains two primer pairs directed towards the genes *chs1* for detection of pan-dermatophytes and *its2* for detection of *T. rubrum*, as shown in table 7. The table also shows the amplicon sizes for the three targets. An Internal Plasmid Control that serves as template for the *T. rubrum* specific primers is added to the Primer Mix as well. All primers are synthetic single-stranded oligonucleotides with free 5'- and 3'-hydroxyl ends. Control 1 consists of *T. mentagrophytes* genomic DNA and control 2 consists of *T. rubrum* genomic DNA.

Table 7. Target detection of the Primer Mix.

| Target detection               | Gene            | Amplicon size (bp) |
|--------------------------------|-----------------|--------------------|
| Pan-dermatophytes <sup>a</sup> | <i>chs1</i>     | 366                |
| <i>T. rubrum</i>               | <i>its2</i>     | 203                |
| Internal Plasmid Control       | Recombinant DNA | ~660               |

<sup>a</sup> Dermatophytes in general e.g. *T. rubrum*, *T. mentagrophytes*, *T. interdigitale*, *T. tonsurans*, *T. schoeuleinii*, *T. violaceum*, *T. soudanense*, *T. verrucosum*, *T. erinacei*, *M. canis*, *M. audouinii*, *E. floccosum* var. *Floccosum*.

## Principle

As for the Dermatophyte Real time PCR Kit (page 6). This conventional PCR based method can detect dermatophytes in general and specifically *T. rubrum*. Using the Dermatophyte conventional PCR Kit the detection can be done within 5 hours.

## Materials Provided

The reagents supplied in the kit are listed in table 8. The kit comprises reagents enough to perform 100 multiplex PCR reactions. Each control vial contains 150 µL corresponding to at least 50 PCR tests.

Table 8. Reagents provided.

| Reagent                        | Cap color | Volume |
|--------------------------------|-----------|--------|
| Primer Mix <sup>a</sup>        | -         | 950 µL |
| Control 1 (pan-derm)           | Blue      | 150 µL |
| Control 2 ( <i>T. rubrum</i> ) | White     | 150 µL |
| PCR ReadyMix <sup>b</sup>      | Red       | 1,2 mL |
| Buffer A                       | -         | 20 mL  |
| Buffer B                       | -         | 20 mL  |

<sup>a</sup>Primer Mix for detection of pan-dermatophytes, *T. rubrum* and Internal Plasmid Control. The plasmid used as template for amplification of the internal control is included in the mix.

<sup>b</sup>Enzyme master mix for conventional PCR. The reagent includes loading buffer.

## Materials and Instruments Required but not Provided

- Tubes for template preparation
- Heat block or water bath (95°C)
- Vortex mixer
- Thermal Cycler
- DNA marker
- 2% agarose gel

The Dermatophyte Conventional PCR Kit has been validated on the instruments: Bio-Rad C1000 and PerkinElmer Cetus.

## Procedure

Template preparation and PCR setup should be performed in dedicated areas free of possible contamination.

### DNA Preparation

1. Add 100 µL of Buffer A to the nail specimen and incubate the sample at 95°C for 10 minutes\*.
2. Immediately add 100 µL of Buffer B and vortex. The sample is ready for PCR.

\*If the nail specimen is large, either cut the nail into small pieces or increase the volume of Buffer A to cover the sample. Increase the volume of Buffer B equally (see item 2).

### PCR Set-up

3. Prepare the total master mix as described in table 9 for the number of samples to be run.
4. Dispense 18 µL of the master mix and 2 µL of template DNA (sample or positive control) in each tube. Prepare a negative control (NTC) by mixing 18 µL of the master mix and 2 µL Buffer A/Buffer B (ratio 1:1).

Table 9. Preparation of the master mix.

| Reagent      | Volume/reaction |
|--------------|-----------------|
| Primer Mix   | 8 µL            |
| PCR ReadyMix | 10 µL           |
| Total volume | 18 µL           |

5. Run the PCR amplification in a Thermal Cycler at the conditions shown in table 10.

Table 10. Amplification protocol.

| Step             | Temp. | Time   | Cycle(s) |
|------------------|-------|--------|----------|
| Pre-denaturation | 94°C  | 5 min  | 1        |
| Denaturation     | 94°C  | 30 sec | 45       |
| Annealing        | 60°C  | 30 sec |          |
| Extension        | 72°C  | 30 sec |          |
| Final extension  | 72°C  | 3 min  | 1        |



6. Run 18 µL of each completed PCR reaction in separate wells on a 2% agarose gel.

Figure 4 shows the PCR results containing a variety of different templates compared to a 100 bp DNA ladder. A *T. rubrum* positive nail specimen will often give a strong band of 203 bp and a weaker or no band at 366 bp due to the relative higher copy number of *its2* compared to *chs1*. Likewise, a positive nail specimen often results in no or a weak internal control band due to relative high concentrations of pan-dermatophyte DNA.

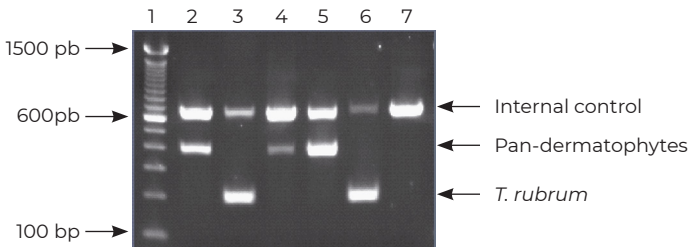


Figure 4. *T. rubrum* specific and pan-dermatophyte multiplex PCR product analysis. Lane 1: Molecular size marker (100 bp DNA ladder); Lane 2: Dermatophyte genomic DNA (Control 1); Lane 3: *T. rubrum* genomic DNA (Control 2); Lane 4: Pan-dermatophyte positive nail specimen (weak); Lane 5: Pan-dermatophyte positive nail specimen (strong); Lane 6: *T. rubrum* positive nail specimen; Lane 7: Negative nail specimen.

## Comparison Study

A total of 118 nail specimens were tested for pan-dermatophytes and *T. rubrum* infection by both the multiplex PCR method and the conventional methods (microscopy and/or culture)<sup>1</sup>. Overall, 42.4% of the specimens were pan-dermatophyte positive by PCR, whereas 38.1% were positive using the conventional methods i.e. the detection of pan-dermatophyte in nail specimens was therefore increased by 4.3% using the multiplex PCR method. Furthermore, the test showed that the detection of *T. rubrum* was increased by 18.6% using the PCR based diagnostic (see table 11).

Table 11. Comparison of pan-dermatophyte and *T. rubrum* detection using the Dermatophyte Conventional PCR kit and conventional methods as microscopy and/or culture.

|                      | <b>Pan-dermatophytes</b> | <b><i>T. rubrum</i></b> |
|----------------------|--------------------------|-------------------------|
| Conventional methods | 38.1%                    | 22.9%                   |
| PCR                  | 42.4%                    | 41.5%                   |
| Increased detection  | 4.3%                     | 18.6%                   |

## Storage and Shelf Life

The Dermatophyte Conventional PCR Kit is shipped with cooling elements but should be stored at -20°C on receipt. Avoid repeated thawing/freezing as it might reduce the stability of the reagents and thus affect the efficiency of the DNA amplification. If you plan to freeze/thaw the primer mix more than 10 times, aliquot out in tubes. The expiry date of the kit is printed on the label.

## E. COLI PCR

### DIARRHOEAGENIC E. COLI PCR KIT (EHEC, EIEC, EPEC AND ETEC)

#### Intended use

The Diarrhoeagenic *E. coli* PCR Kit (EHEC, EIEC, EPEC and ETEC) is for *in vitro* diagnostic PCR detection of diarrheagenic *E. coli* (DEC)<sup>2</sup>. The kit detects four important diarrheagenic *E. coli* groups that are pathogenic for humans: EHEC, EIEC, EPEC and ETEC<sup>3</sup>.

#### Description

The kit contains: Primer Mix, two PCR positive DNA controls, PCR ReadyMix (including loading buffer), TE-Buffer (10 mM Tris-HCL, 1 mM EDTA, pH 8) and 10% Chelex-100 in TE-Buffer (magnetic stir bar added). The Primer Mix contains 8 primer pairs directed towards the following genes: *eae* (intimin), *vtx1* (verocytotoxin 1), *vtx2* (verocytotoxin 2), *estA-human* (heat stable enterotoxin human variant), *estA-porcine* (heat stable enterotoxin porcine variant), *eltA* (heat labile enterotoxin), *ipaH* (invasive plasmid antigen) and *16S* rDNA (internal positive control).

DNA Control 1 consists of purified DNA from a non-pathogenic *E. coli* plus DNA from a strain with the *eae*, *vtx1* and *vtx2* genes. DNA Control 2 contains a mixture of purified DNA from a non-pathogenic *E. coli* strain, a strain with the *ipaH* gene and a strain with the *eltA* and *estA* genes. Together, the two controls represent all 7 virulence genes and the Internal Positive Control (16S rDNA). Table 12 on page 20 shows the combination of virulence genes as well as the amplicon size of the fragments for the two controls.

Table 12. Virulence genes and amplicon sizes detected in DNA Control 1 and 2.

| DNA Control 1 | DNA Control 2        | Amplicon size (bp) |
|---------------|----------------------|--------------------|
| 16S rDNA      | 16S rDNA             | 1062               |
|               | <i>ipaH</i>          | 647                |
|               | <i>eltA</i>          | 479                |
| <i>vtx2</i>   |                      | 420                |
| <i>eae</i>    |                      | 377                |
| <i>vtx1</i>   |                      | 260                |
|               | <i>estA</i> -porcine | 160                |
|               | <i>estA</i> -human   | 151                |

## Principle

The kit detects four important DEC groups: EHEC, EIEC, EPEC and ETEC. The genes used for identification of the particular DEC groups are listed in table 13.

Table 13. Virulence genes and identified DEC group.

| Gene                     | DEC                      |
|--------------------------|--------------------------|
| 16S rDNA <sup>a</sup>    | -                        |
| <i>ipaH</i> <sup>b</sup> | EIEC                     |
| <i>eltA</i>              | ETEC                     |
| <i>vtx2</i>              | EHEC                     |
| <i>eae</i>               | EPEC, A/EEC <sup>c</sup> |
| <i>vtx1</i> <sup>d</sup> | EHEC                     |
| <i>estA</i> -porcine     | ETEC                     |
| <i>estA</i> -human       | ETEC                     |

<sup>a</sup> The 16S rDNA primers included in the Primer Mix are designed to detect most Gram-negative bacteria, allowing an evaluation of the PCR.

<sup>b</sup> Might also be present in *Shigella* spp.

<sup>c</sup> EPEC and A/EEC distinction is based on serotype.

<sup>d</sup> Might also be present in *Shigella dysenteriae* I.

## Materials Provided

The reagents supplied in the kit are listed in table 14. The kit comprises reagents enough to perform 100 multiplex PCR reactions. Each control vial contains 150 µL corresponding to at least 25 PCR tests.

Table 14. Reagents provided.

| Reagent                   | Cap color | Volume |
|---------------------------|-----------|--------|
| Primer Mix <sup>a</sup>   | Blue      | 700 µL |
| DNA Control 1             | White     | 150 µL |
| DNA Control 2             | Red       | 150 µL |
| PCR ReadyMix <sup>b</sup> | -         | 1,2 mL |
| 10% Chelex-100            | -         | 25 mL  |
| TE-Buffer                 | -         | 15 mL  |

<sup>a</sup> Primer Mix for detection of the DEC groups EHEC, EIEC, EPEC and ETEC.

<sup>b</sup> Including loading buffer.

## Materials and Instruments Required but not Provided

- Selective agar plates
- Tubes for template preparation
- Tube cap locks
- DNA Marker
- 2% agarose gel
- Incubator (35-37°C)
- Heat block or water bath (95°C)
- Vortex Mixer
- Thermal Cycler

The Diarrhoeagenic *E. coli* PCR Kit (EHEC, EIEC, EPEC AND ETEC) has been validated on the following instruments: Bio-Rad C1000, Applied Biosystems 9600 and 9700.

## Precautions

It is not possible to use the stool sample directly in the PCR reaction.

## Procedure

### DNA preparation

DNA preparation and PCR setup should be performed in dedicated areas free of possible contamination.

1. Plate the stool sample on a selective agar plate over night at 35-37°C.
2. Place the bottle with 10% Chelex-100 on a magnetic stirrer and pipette

200 µL/tube while the Chelex-100 is homogeneous. Pick 5-10 plate-grown colonies (both lactose positive and negative) and suspend them in the aliquoted 10% Chelex-100.

3. Boil the suspension for 5 min. (remember tube cap locks) and centrifuge briefly (5 min. at approx. 2200g).
4. Dilute 15 µL of the supernatant in 100 µL TE-Buffer and use 4 µL directly in the PCR.

PCR set-up

5. Prepare the master mix as described in table 15 for the number of samples to be run.

Table 15. Preparation of the master mix.

| Reagent      | Volume/reaction |
|--------------|-----------------|
| PCR ReadyMix | 10 µL           |
| Primer Mix   | 6 µL            |
| Total        | 16 µL           |

6. Dispense 16 µL of the master mix and 4 µL of template DNA (sample or positive control) in each tube and mix. Prepare a negative control (NTC) by mixing 16 µL of the master mix and 4 µL TE Buffer.

7. Run the PCR amplification in a Thermal Cycler as described in table 16.

Table 16. Amplification protocol

| Step             | Temp. | Time   | Cycle(s) |
|------------------|-------|--------|----------|
| Pre-denaturation | 95°C  | 2 min  | 1        |
| Denaturation     | 94°C  | 50 sec | 35       |
| Annealing        | 62°C  | 40 sec |          |
| Extension        | 72°C  | 50 sec |          |
| Final extension  | 72°C  | 3 min  | 1        |

8. Run 18 µL of each completed PCR reaction in separate wells on a 2% agarose gel capable of separating the particular amplicon sizes.

# Interpretation of the Analysis Results

Figure 5 shows the result of PCR reactions containing a variety of different templates. The amplified fragments are compared to a 100 bp DNA marker.

If very high template concentrations are used in the PCR analysis of EIEC strains, an unspecific fragment of ~750 bp may be amplified in addition to the 647 bp long *ipaH* fragment. The intensities of the two bands depend on the template concentration. Dilution of the template may therefore prevent amplification of the unspecific band.

A DEC positive sample giving a strong band for one or more of the virulence genes will often result in a weak internal control band (not shown on the gel below).

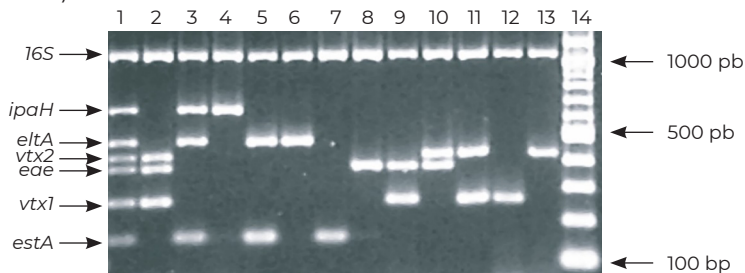


Figure 5. Analysis of 13 different templates.

Lane 1: Shows all fragments and the corresponding virulence genes (written to the left side of the figure) that can be detected by the kit, lane 2: DNA Control 1: *vtx2*, *eae* and *vtx1*, lane 3: DNA Control 2: *ipaH*, *eltA* and *estA*, lane 4: *ipaH*, lane 5: *eltA* and *estA*, lane 6: *eltA*, lane 7: *estA*, lane 8: *eae*, lane 9: *eae* and *vtx1*, lane 10: *vtx2* and *eae*, lane 11: *vtx2* and *vtx1*, lane 12: *vtx1* and lane 13: *vtx2*. Lane 14 is a 100 bp DNA marker.

## Comparison Study

A total of 142 reference strains from “The National *Escherichia* and *Klebsiella* Centre”, Statens Serum Institut, Denmark were tested by the multiplex PCR method. The strains represented a broad variety of different serotypes and virulence gene combinations. Compared to a DNA hybridisation technique targeting the same genes, the PCR method showed 100% agreement. Also tested were 13 non-*E. coli* species, all of which only produced the 16S rDNA control band as expected.

## Storage and Shelf Life

The Diarrhoeagenic *E. coli* PCR Kit (EHEC, EIEC, EPEC and ETEC) is shipped with cooling elements but should be stored at the following conditions on receipt: The DEC primer mix, the PCR ReadyMix and the DNA controls should be stored at -20°C. If running only a few PCR reactions at a time, dispense the primer mix and the PCR ReadyMix into several aliquots. The DEC primer mix and the controls in use can be stored at 2-8°C for up to 2 weeks. Store 10% Chelex-100 and TE-Buffer at room temperature. The expiry date of the kit is printed on the label.



# ENTEROAGGREGATIVE *E. COLI* PCR KIT (EAEC)

## Intended use

The Enteroaggregative *E. coli* PCR Kit is for *in vitro* diagnostic PCR detection of enteroaggregative *E. coli* (EAEC). The kit is a supplement to the Diarrhoeagenic *E. coli* PCR Kit (page 19) and PCR reactions should be performed using a template prepared according to the description on page 21-22. PCR reactions should also be performed under the same Thermal Cycler conditions as the Diarrhoeagenic *E. coli* PCR kit, i.e. the Diarrhoeagenic *E. coli* PCR and the Enteroaggregative *E. coli* PCR reactions can run in the Thermal Cycler simultaneously.

## Description

The kit contains: Primer Mix, a PCR positive DNA control and PCR ReadyMix (including loading buffer).

The Primer Mix contains 4 primer pairs directed towards the following genes: *aatA* (dispersin transporter protein), *aggR* (Transcriptional activator), *aaiC* (AaiC, secreted protein) and 16S rDNA (Positive Internal Control). All primers are synthetic single-stranded oligonucleotides with free 5'- and 3'- hydroxyl ends. The individual concentration of each primer pair is adjusted for optimal performance in the multiplex PCR.

The PCR positive control consists of purified DNA from a non-pathogenic *E. coli* plus DNA from a strain with the *aatA*, *aggR* and *aaiC* gene. Table 17 shows the combination of virulence genes and the amplicon size of the fragments for the DNA Control.

Table 17. Virulence genes and amplicon size detected in the DNA Control.

| DNA Control           | Amplicon size (bp) |
|-----------------------|--------------------|
| 16S rDNA <sup>a</sup> | 1062               |
| <i>aatA</i>           | 550                |
| <i>aggR</i>           | 323                |
| <i>aaiC</i>           | 214                |

<sup>a</sup>The 16S rDNA primers included in the Primer Mix are designed to detect most Gram-negative bacteria, allowing an evaluation of the PCR.

## Principle

Virulence gene *aatA*, *aggR* and *aaiC* determine all three the EAEC group. Different combinations of the three genes appear. Identification of either one, two or all three virulence genes in an *E. coli* culture determine the EAEC group.

## Materials Provided

The reagents supplied in the kit are listed in table 18.

The kit contains reagents enough to perform 100 multiplex PCR reactions.

The DNA Control vial contains 150 µL corresponding to at least 25 PCR tests.

Table 18. Reagents provided

| Reagent                   | Cap color | Volume |
|---------------------------|-----------|--------|
| Primer Mix <sup>a</sup>   | Blue      | 700 µL |
| DNA Control               | White     | 150 µL |
| PCR ReadyMix <sup>b</sup> | Red       | 1,2 mL |

<sup>a</sup>Primer Mix for detection of the DEC group EAEC.

<sup>b</sup>Including loading buffer.

## Materials and Instruments Required but not Provided

- Selective agar plate
- 10% chelec-100 (included in the Diarrhoeagenic *E. coli* PCR Kit, page 19)
- TE buffer (included in the Diarrhoeagenic *E. coli* PCR Kit, page 19)
- Tubes for template preparation
- Tube cap locks

- 2% agarose gel
- DNA marker
- Incubator (35-37°C)
- Heat block or water bath (95°C)
- Vortex Mixer
- Thermal Cycler

The Enteraggregative *E. coli* PCR Kit has been validated on the instrument: Bio-Rad C1000.

## Precautions

It is not possible to use the stool sample directly in the PCR reaction.

## Procedure

The DNA preparation, the PCR set-up and the separation of the amplicon sizes should be performed as described for The Diarrhoeagenic *E. coli* PCR Kit on page 19-24.

## Interpretation of the Analysis Results

Figure 6 shows the results of PCR reactions containing a variety of different templates compared to a 100 bp DNA marker. Identification of one, two or all three of the virulence genes (*aatA*, *aggR*, *aaiC*) in an *E. coli* culture determine the EAEC group.

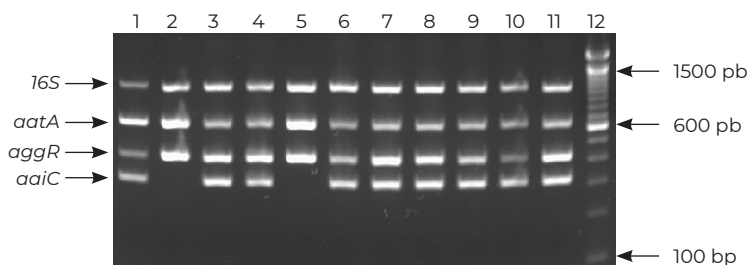


Figure 6: Analysis of 11 different templates.

Lane 1: DNA control (*16S*, *aatA*, *aggR* and *aaiC*), lane 2: *aatA* and *aggR*, lane 3 and 4: *aatA*, *aggR* and *aaiC*, lane 5: *aatA* and *aggR*, lane 6- 11: *aatA*, *aggR* and *aaiC*, 12: 100 bp DNA marker.

## Comparison Study

A total of 72 EAEC strains from “The National *Escherichia* and *Klebsiella* Centre”, Statens Serum Institut, Denmark, have been tested with the Enteroaggregative *E. coli* PCR Kit. Compared to results obtained by doing PCR, sequencing, and/or DNA hybridisation technique at “The National *Escherichia* and *Klebsiella* Centre” the kit showed 99% agreement.

## Storage and Shelf Life

Enteroaggregative *E. coli* PCR Kit (EAEC) is shipped with cooling elements but should be stored at -20°C on receipt. If running only a few PCR reactions at a time, dispense the primer mix and the PCR ReadyMix into several aliquots and store them at -20°C. The Primer Mix and the DNA Control in use can be stored at 2-8°C for up to 2 weeks. The expiry date of the kit is printed on the label.

# DIARRHOEAGENIC E. COLI PCR KIT (EHEC, EIEC, EPEC, ETEC AND EAEC)

## Intended use

The Diarrhoeagenic *E. coli* PCR Kit (EHEC, EIEC, EPEC, ETEC and EAEC) is for *in vitro* diagnostic PCR detection of diarrhoeagenic *E. coli* (DEC)<sup>2</sup>. The kit detects the 5 most important diarrhoeagenic groups that are pathogenic for humans; EHEC, EIEC, EPEC, ETEC and EAEC<sup>3</sup>.

## Description

The kit contains: Primer Mix, two PCR positive DNA controls, PCR ReadyMix (including loading buffer), TE-Buffer (1 mM Tris-HCL, 1 mM EDTA, pH 8) and 100 µl Chelex-100 in TE-Buffer (magnetic stir bar added). The Primer Mix amplifies a specific fragment of the following genes: *eae* (intimin), *vtx1* (verocytotoxin 1), *vtx2* (verocytotoxin 2), *estA-human* (heat stable enterotoxin human variant), *estA-porcine* (heat stable enterotoxin porcine variant), *eltA* (heat labile enterotoxin), *ipaH* (invasive plasmid antigen), *aatA* (dispersin transporter protein), *aggR* (transcriptional activator), *aaiC* (AaiC secreted protein) and *16S* rDNA (internal positive control). All primers are synthetic single-stranded oligonucleotides with free 5'- and 3'- hydroxyl ends. The individual concentration of each primer pair is adjusted for optimal performance in the multiplex PCR.

The two positive PCR controls, DNA Control 1 and 2, consist of purified DNA from different DEC strains. Together, the two controls represent all 10 virulence genes and the internal positive control (*16S* rDNA). Table 19 shows the combination of virulence genes as well as the amplicon size of the fragments for the two controls.

Table 19. Virulence genes and amplicon size detected in DNA Control 1 and 2

| Control 1            | Control 2          | Amplicon size (bp) |
|----------------------|--------------------|--------------------|
| 16S rDNA             | 16S rDNA           | 1062               |
| <i>ipaH</i>          |                    | 647                |
|                      | <i>aatA</i>        | 550                |
|                      | <i>eltA</i>        | 479                |
| <i>vtx2</i>          |                    | 420                |
| <i>Eae</i>           |                    | 377                |
|                      | <i>aggR</i>        | 323                |
| <i>vtx1</i>          |                    | 260                |
|                      | <i>aaiC</i>        | 217                |
| <i>estA</i> -porcine |                    | 161                |
|                      | <i>estA</i> -human | 151                |

## Principle

The kit detects the five most important DEC groups: EHEC, EIEC, EPEC, ETEC and EAEC. The genes used for identification of the particular DEC groups are listed in table 20.

Table 20. Virulence genes and identified DEC group.

| Gene                              | DEC                      |
|-----------------------------------|--------------------------|
| 16S rDNA <sup>a</sup>             | -                        |
| <i>ipaH</i> <sup>b</sup>          | EIEC                     |
| <i>aatA</i>                       | EAEC                     |
| <i>eltA</i>                       | ETEC                     |
| <i>vtx2</i>                       | EHEC                     |
| <i>eae</i>                        | EPEC, A/EEC <sup>c</sup> |
| <i>aggR</i>                       | EAEC                     |
| <i>vtx1</i> <sup>d</sup>          | EHEC                     |
| <i>aaiC</i>                       | EAEC                     |
| <i>estA</i> -porcine <sup>e</sup> | ETEC                     |
| <i>estA</i> -human <sup>e</sup>   | ETEC                     |

<sup>a</sup> The 16S rDNA primers included in the Primer Mix are designed to detect most Gram-negative bacteria, allowing an evaluation of the PCR.

<sup>b</sup> Might also be present in *Shigella* spp.

<sup>c</sup> EPEC and A/EEC distinction is based on serotype.

<sup>d</sup> Might also be present in *Shigella dysenteriae* 1.

<sup>e</sup> Amplicon sizes for *estA*-porcine and *estA*-human are very similar.

### Materials provided

The reagents supplied in the kit are listed in table 21. The kit comprises reagents enough to perform 100 multiplex PCR reactions. Each Control vial contains 150 µL corresponding to at least 25 PCR tests.

Table 21. Reagents provided.

| Reagent                   | Cap color | Volume |
|---------------------------|-----------|--------|
| Primer Mix <sup>a</sup>   | Blue      | 700 µL |
| DNA Control 1             | White     | 150 µL |
| DNA Control 2             | Red       | 150 µL |
| PCR ReadyMix <sup>b</sup> | -         | 1 mL   |
| 10% Chelex-100            | -         | 2 mL   |
| TE-Buffer                 | -         | 15 mL  |

<sup>a</sup>Primer Mix for detection of the EEC groups EHEC, EIEC, EPEC, ETEC and EAEC.

<sup>b</sup>Including loading buffer.

### Materials and Instruments Required but not Provided

- Agar plates
- Inoculation loops
- Tubes for template preparation
- Tube cap locks
- DNA marker
- 2% agarose gels
- Incubator (35-37°C)
- Heat block or water bath (95°C)
- Vortex Mixer
- Thermal Cycler

The Diarrhoeagenic E. coli PCR Kit (EHEC, EIEC, EPEC, ETEC and EAEC) has been validated on the instrument: Bio-Rad C1000.

### Precautions

It is not possible to use the stool sample directly in the PCR reaction.

## Procedure

The DNA preparation, the PCR set-up and the separation of the amplicon sizes should be performed as described for the Diarrhoeagenic *E. coli* PCR Kit on page 19-24.

## Interpretation of the Analysis Results

Figure 7 shows the results of PCR reactions containing a variety of different templates compared to a 100 bp DNA marker. For further details please see description for the Diarrhoeagenic *E. coli* PCR Kit on page 23.

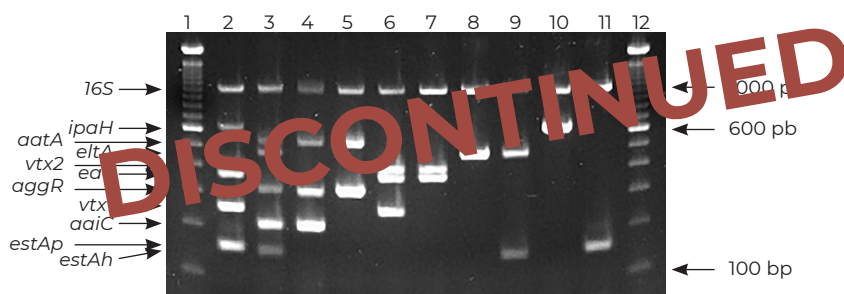


Figure 7: Analysis of 10 different templates.

Lane 1: Shows all fragments and the corresponding virulence genes (written to the left side of the figure) that can be detected by the kit, lane 2 (DNA Control 1): *ipaH*, *vt2*, *eae*, *vt1* and *estA*-porcine, lane 3 (DNA Control 2): *aatA*, *eltA*, *aggR*, *aaiC* and *estA*-human, lane 4: *aatA*, *aggR* and *aaiC*, lane 5: *aatA* and *aggR*, lane 6: *vt2*, *eae* and *vt1*, lane 7: *vt2* and *eae*, lane 8: *eltA*, lane 9: *eltA* and *estA*-human, lane

10: *ipaH*, lane 11: *estA*-porcine, lane 12: 100 bp DNA marker.

## Comparison Study

The Diarrhoeagenic *E. coli* PCR Kit (EHEC, EIEC, EPEC, ETEC and EAEC) has been tested with a panel of 161 *E. coli* (reference strains and clinical isolates) from "The National *Escherichia* and *Klebsiella* Centre", Statens Serum Institut, Denmark. The panel represented a broad variety of different serotypes and virulence gene combinations.

Compared to results obtained by doing PCR, sequencing, and/ or DNA hybridisation technique at "The National *Escherichia* and *Klebsiella* Centre" the kit showed 99% agreement. 14 non-*E. coli* species were also tested, all of which only produced the 16S rDNA control band, as expected.



## Storage and Shelf Life

The Diarrhoeagenic *E. coli* PCR Kit (EHEC, EIEC, EPEC, ETEC and EAEC) is shipped with cooling elements but should be stored at the following conditions on receipt: The DEC primer mix, the PCR ReadyMix and the DNA controls should be stored at -20°C. If running only a few PCR reactions at a time, dispense the primer mix and the PCR ReadyMix into several aliquots and store them at -20°C. The DEC primer mix and the controls in use can be stored at 2-8°C for up to 2 weeks. Store 10% Chelex-100 and TE-Buffer at room temperature. The expiry date of the kit is printed on the label.

**DISCONTINUED**

# DIARRHOEAGENIC E. COLI PRIMER MIX

## (EHEC, EIEC, EPEC AND ETEC)

### Intended use

The Diarrhoeagenic *E. coli* Primer Mix (EHEC, EIEC, EPEC and ETEC) is for *in vitro* diagnostic PCR detection of diarrhoeagenic *E. coli* (DEC)<sup>2</sup>. The kit detects four important diarrhoeagenic *E. coli* groups that are pathogenic for humans: EHEC, EIEC, EPEC and ETEC<sup>3</sup>.

### Description

The product contains Primer Mix and two positive DNA Controls. The Primer mix contains the same primers as described for the Diarrhoeagenic *E. coli* PCR Kit on page 19. Likewise, DNA Control 1 and 2 consist of DNA from the same *E. coli* strains as described on page 19. Virulence genes detected and amplicon sizes are shown in table 12 on page 20.

### Principle

As for the Diarrhoeagenic *E. coli* PCR Kit (page 20).

### Materials provided

The reagents supplied in the kit are listed in table 22. The kit comprises reagents enough to perform 100 multiplex PCR reactions when using 4 µL of the Primer Mix per reaction.

Table 22. Reagents provided.

| Reagent                 | Cap color | Volume |
|-------------------------|-----------|--------|
| Primer Mix <sup>a</sup> | Blue      | 520 µL |
| DNA Control 1           | White     | 1 mL   |
| DNA Control 2           | Red       | 1 mL   |

<sup>a</sup>Primer Mix for detection of the DEC groups EHEC, EIEC, EPEC and ETEC.

### Materials and Instruments required but not provided

Other reagents than the ones shown below may be used, but as the thermocycler conditions are optimized with the listed reagents. Changes may require additional optimization.

- Selective agar plates
- Tubes for template preparation
- TE-Buffer (10mM Tris-HCl, 1mM EDTA, pH 8)
- 10% Chelex-100 in TE-Buffer
- Tube cap locks
- dNTP-mix (each 1.25 mM)
- 50 mM MgCl<sub>2</sub>
- PCR grade water
- Platinum $\rightarrow$ Taq Polymerase 5 U/ $\mu$ L (Invitrogen)
- 10 x PCR buffer (200 mM Tris-HCl (pH 8.4), 500 mM KCl)
- DNA Marker
- Loading Buffer
- 2% agarose gel
- Incubator (35-37°C)
- Heat block or water bath (95°C)
- Vortex Mixer
- Thermal Cycler

The Diarrhoeagenic *E. coli* Primer Mix (EHEC, EIEC, EPEC and ETEC) has been validated on the following instruments: Bio-Rad C1000, MJ Research Tetrad 2 and Applied Biosystems 9600 and 9700.

## Precautions

It is not possible to use the stool sample directly in the PCR reaction.

## Procedure

### DNA preparation

DNA preparation and PCR setup should be performed in dedicated areas free of possible contamination.

1. Plate the stool sample on a selective agar plate over night at 35-37°C.
2. Pick 5-10 plate-grown colonies (both lactose positive and negative) and suspend them in a tube containing 200  $\mu$ L 10% Chelex-100 dissolved in

TE-buffer.

3. Boil the suspension for 5 min. (remember tube cap locks) and centrifuge briefly (5 min. at app. 2200g).
4. Dilute 15  $\mu\text{L}$  of supernatant in 100  $\mu\text{L}$  1xTE-buffer and use 8  $\mu\text{L}$  directly in the PCR (other commercial DNA extraction methods may also be used).

#### PCR set-up

1. Prepare the master mix as described in table 23 for the number of samples to be run.

Table 23. Preparation of the master mix

| Reagents                 | Volume/reaction    |
|--------------------------|--------------------|
| PCR grade water          | 11.1 $\mu\text{L}$ |
| 10 x PCR Buffer          | 5.0 $\mu\text{L}$  |
| 50 mM Mg Cl <sub>2</sub> | 3.5 $\mu\text{L}$  |
| dNTP-mix (each 1.25 mM)  | 8.0 $\mu\text{L}$  |
| Primer Mix               | 4.0 $\mu\text{L}$  |
| Total volume             | 31.6 $\mu\text{L}$ |

2. Dispense 31.6  $\mu\text{L}$  of the master mix and 8  $\mu\text{L}$  of template DNA (sample or positive control) in each tube. Prepare a negative control (NTC) by mixing 31.6  $\mu\text{L}$  of the master mix and 8  $\mu\text{L}$  TE Buffer.
3. At last add 0.40  $\mu\text{L}$  Platinum<sup>+</sup>Taq Polymerase 5 U/ $\mu\text{L}$  to each tube and mix.
4. Run the PCR amplification in a Thermal Cycler as described in table 24.

Table 24. Amplification protocol.

| Step             | Temp. | Time   | Cycle(s) |
|------------------|-------|--------|----------|
| Pre-denaturation | 95°C  | 2 min  | 1        |
| Denaturation     | 94°C  | 50 sec | } 35     |
| Annealing        | 62°C  | 40 sec |          |
| Extension        | 72°C  | 50 sec |          |
| Final extension  | 72°C  | 3 min  | 1        |

5. Add loading buffer to each tube and run 18  $\mu\text{L}$  of the completed PCR reactions in separate wells on an agarose gel (2%) capable of separating the particular amplicon sizes.

# Interpretation of the Analysis Results

Figure 8 shows the results of PCR reactions containing a variety of different templates compared to a 100 bp DNA marker.

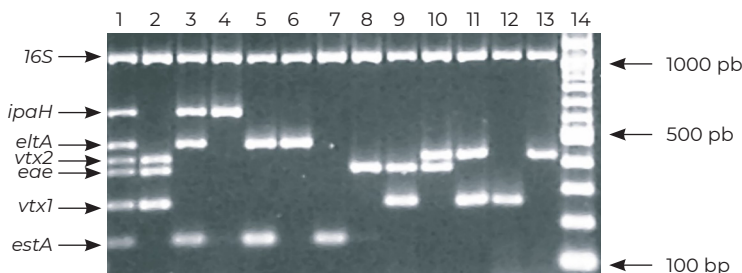


Figure 8. Analysis of 13 different templates.

Lane 1: Shows all fragments and the corresponding virulence genes (written to the left side of the figure) that can be detected by the kit, lane 2: DNA Control 1: *vtx2*, *eae* and *vtx1*, lane 3: DNA Control 2: *ipaH*, *eltA* and *estA*, lane 4: *ipaH*, lane 5: *eltA* and *estA*, lane 6: *eltA*, lane 7: *estA*, lane 8: *eae*, lane 9: *eae* and *vtx1*, lane 10: *vtx2* and *eae*, lane 11: *vtx2* and *vtx1*, lane 12: *vtx1* and lane 13: *vtx2*. Lane 14 is a 100 bp DNA marker.

## Comparison Study

As described for the Diarrhoeagenic *E. coli* PCR Kit (EHEC, EIEC, EPEC and ETEC) on page 23.

## Storage and Shelf Life

The Diarrhoeagenic *E. coli* Primer Mix (EHEC, EIEC, EPEC and ETEC) is shipped with cooling elements but should be stored at -20°C on receipt. If running only a few PCR reactions at a time, dispense the Primer Mix into several aliquots and store it at -20°C. The Primer Mix and the controls in use can be stored at 2-8°C for up to 2 weeks. The expiry date of the kit is printed on the label.

## REFERENCES

1. Brillowska-Dabrowska, A., Saunte, D. M. and Arendrup, M. C. 2007. Five-Hour Diagnosis of Dermatophyte Nail Infection with Specific Detection of *Trichophyton rubrum*. Jour. Clin. Microbiol. 1200-1204.
2. Persson, S., Olsen, K. E. P., Scheutz, F., Krogfelt, K. A. and Gerner-Smidt, P. 2007. A method for fast and simple detection of major diarrhoeagenic *Escherichia coli* in the routine diagnostic laboratory. Clin Microbiol Infect. 13:516-524.
3. Nataro, J. P. and J. B. Kaper. 1998. Diarrhoeagenic *Escherichia coli*. Clin. Microbiol Rev. 11:142-201.

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