For use in quality control / manufacturing process only.

QC Sample Preparation Kit

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Preparation of cell culture samples for PCR tests to detect mycoplasma or residual DNA from CHO cells and from *E. coli*.

Cat. No. 08 146 829 001 1 kit

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	orange	Proteinase K	–	4 vials, 850 µl each
2	brown	Lysis Buffer	Contains guanidine thiocyanate, Tris buffer, and detergent.	5 vials, 6 ml each
3	grey	Precipitation Reagent	To precipitate nucleic acids.	5 vials, 7 ml each
4	white	Washing Buffer	For washing steps.	6 vials, 9 ml each
5	blue	Dissolution Buffer	To dissolve the extracted nucleic acids.	5 vials, 5 ml each
6	white	Water, DNA free	–	10 vials, 1 ml each
7	white	Poly(A)	Lyophilized	7 bottles, 2 mg each
8	–	Reaction vials	–	3 bags with 35 polypropylene tubes (2 ml)

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the kit is stable through the expiry date printed on the label.

Vial / bottle	Cap	Label	Storage
1	orange	Proteinase K	Once opened, store all kit components at +15 to +25°C. ⚠ ***Do not store at +2 to +8°C or −15 to −25°C.***
2	brown	Lysis Buffer	
3	grey	Precipitation Reagent	
4	white	Washing Buffer	
5	blue	Dissolution Buffer	
6	white	Water, DNA free	
7	white	Poly(A)	
8	–	Reaction vials	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free, DNA-free, aerosol-resistant pipette tips
- Alcohol wipes
- Biosafety cabinet class II
- Thermomixer
- Benchtop centrifuge (for 2 ml tubes)
- Vortex mixer

For preparation of working solution

- Residual DNA *E. coli* Kit*, or
- Residual DNA CHO Kit*

For detection of mycoplasma contamination

- Thermal block cycler and the MycoTOOL Mycoplasma Detection Amplification Kit*, or
- Real-time PCR instrument and the MycoTOOL Real-Time PCR Kit*

For detection of host cell DNA

- Residual DNA *E. coli* Kit*, or
- Residual DNA CHO Kit*

1.4. Application

The QC Sample Preparation Kit is designed for:
- 26 extractions from mammalian cell culture samples (per 1 ml), or
- 10 extractions from high density cell culture samples (per 1 ml), or
- 100 extractions from fermentation process samples (per 100 µl).

The kit offers optimized extraction protocols to prepare samples for further PCR tests, such as detection of mycoplasma contamination and of host cell DNA.

Detection of mycoplasma contamination

Using a PCR block cycler, refer to the Instructions for Use of the MycoTOOL Mycoplasma Detection Amplification Kit*.
- For testing of CHO or SP2/0 cells with 5 × 10^6 cells/ml (standard protocol).
- For testing of CHO or SP2/0 samples up to 1 × 10^8 cells/ml (protocol for high cell density).

Using a real-time PCR instrument, refer to the Instructions for Use of the MycoTOOL Real-Time PCR Kit* for testing of mammalian cell culture samples.

This PCR kit contains a control plasmid (Recovery Control) that can be added to the sample material to prevent false-negative results.

Detection of host cell DNA

The QC Sample Preparation Kit is suitable for processing samples from biopharmaceutical production with host cell impurities from fermentation process to detect residual DNA from *E. coli* or CHO cells.
Refer to the Instructions for Use of the Residual DNA *E. coli* Kit* or the Residual DNA CHO Kit*.

These PCR kits contain a workflow negative control to prevent false-negative results and a Standard DNA that can be added to the sample material.

1.5. Preparation Time

Hands-on time: approximately 2 hours.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Precautions

To avoid contamination, perform the workflow setup under DNA-free conditions.
- Prepare and pipette all solutions with nuclease-free, DNA-free equipment and consumables.
- UV-treat the laminar flow hood prior to pipetting.
- Use sterile, single-use gloves and freshly laundered laboratory coats.
- Close vials immediately after pipetting.
- Segregate sequential workflow steps:

Room	Workflow Step
Sample preparation	Extraction and purification of test samples, including preparation of recovery control sample.
Master mix preparation	▪ Master mix preparation. ▪ Pipetting of PCR Negative Control to the NTC wells.
PCR for setup and amplification run	▪ Dilution and pipetting of samples and PCR Positive Control to the PCR plate. ▪ Running the LightCycler® 480 Instrument II.

Safety Information

Precautions

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material can vary, the operator must optimize pathogen inactivation and follow the appropriate measures according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online at documentation.roche.com, or upon request from the local Roche office.

2.2. Protocols

Experimental overview of sample preparation

The following table describes the workflows to prepare DNA from cell culture samples.

⚠ ***Careful pipetting is important for consistent results. Ensure that no extra solution is on the outside of the pipette tip before liquid transfer. When adding a solution to the tubes, immerse the tip into the reaction mixture, deliver the solution slowly, and then remove the tip by sliding it up the wall of the vessel.***

Workflow A	Workflow B	Workflow C	Workflow D (optional)
Sample	Workflow negative control[(1)] or buffer	Workflow negative control[(1)] + DNA Standard or Internal control[(1)]	Sample + DNA Standard or Internal control[(1)]
↓	↓	↓	↓
Sample preparation	Sample preparation	Sample preparation	Sample preparation
↓	↓	↓	↓
Eluted DNA	Eluted DNA (workflow negative control)	Eluted DNA (workflow positive control)	Eluted DNA (recovery control)

[(1)] Vials included in the PCR Kit.

Preparation of DNA sample from cell culture

The following steps describe only a general overview.

ⓘ *Refer to the specific PCR kits for detailed information about pre-treatment of samples, volume of solution to be added, and incubation time.*

1. Equilibrate the thermomixer to +56°C.

2. Prepare the samples according to the Instructions for Use of the corresponding PCR Kit.
   – Prepare the appropriate number of Reaction vials by adding 30 to 50 µl Proteinase K (Vial 1) each.
   – Label the Reaction vials accordingly.

3. Add 200 to 450 µl of sample to each Reaction vial.

4. Add 220 to 700 µl Lysis Buffer (Vial 2) or working solution[(1)] (Poly A + Lysis Buffer) to each Reaction vial.

5. Close the Reaction vials and vortex 3 times for 5 seconds.

6. Incubate for 15 to 30 minutes at +56°C and 600 to 900 rpm in the thermomixer.

7. Remove the Reaction vials.
   – Equilibrate the thermomixer to +80°C for the next incubation step.

8. Add 290 to 800 µl Precipitation Reagent (Vial 3) to each Reaction vial.
   – Close the Reaction vials, invert 20 times, and vortex for 5 seconds.

9. Centrifuge for 3 minutes at 16,000 × *g*.
   – Decant supernatant without removing pellet.

10. Add 450 to 1,000 µl Washing Buffer (Vial 4).

11. Close the Reaction vials and invert 5 times.
    – Centrifuge immediately for 3 minutes at 16,000 × *g* and carefully remove all of the supernatant.

12 Centrifuge briefly for 3 seconds at 16,000 × *g* and carefully remove the residual supernatant.

13 Add 100 to 600 µl[1] Dissolution Buffer (Vial 5).

14 Close the Reaction vials and dissolve the pellet for 10 to 30 minutes at +80°C and 900 to 1,300 rpm in the thermomixer.

15 Vortex until the pellet is completely dissolved.

16 Transfer the Reaction vials to the PCR room.

⚠ ***Eluted DNA is stable for 3 days at −15 to −25°C.***

[1] For the preparation of the working solution, refer to the Instructions for Use of the Residual DNA *E. coli* Kit* or the Residual DNA CHO Kit*.

3. Additional Information on this Product

3.1. Test Principle

As a prerequisite for the analysis of samples by PCR, the isolation of the analyte from the cell culture is required.

① Cell lysis is accomplished by incubation of the sample in a special lysis buffer in the presence of Proteinase K.

② Subsequently, the DNA is precipitated and purified from salts, proteins, and other impurities by a washing step.

③ In a final step, the DNA is dissolved in a low-salt buffer.

3.2. Quality Control

Each lot of the QC Sample Preparation Kit is function tested for the detection of residual host cell CHO DNA and mycoplasma with the MycoTOOL Mycoplasma Real-Time PCR Kit*.
All reagents of the QC Sample Preparation Kit are tested for the absence of DNA from mycoplasma, CHO cells, and *E. coli*.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Instruments		
LightCycler® 480 Instrument II	1 instrument	05 015 278 001
	1 instrument	05 015 243 001
Reagents, kits		
MycoTOOL Mycoplasma Detection Amplification Kit	1 kit	05 184 240 001
MycoTOOL Mycoplasma Real-Time PCR Kit	1 kit, 160 PCRs each with a 50 µl final reaction volume	06 495 605 001
Residual DNA *E. coli* Kit	1 kit, 96 reactions	07 728 735 001
Residual DNA CHO Kit	1 kit, 96 PCRs each with a 20 µl final reaction volume	07 427 689 001

4.4. Trademarks

MYCOTOOL and LIGHTCYCLER are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
http://documentation.roche.com.

4.6. Regulatory Disclaimer

For use in quality control / manufacturing process only.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

For additional documentation such as certificates and safety data sheets, please visit **documentation.roche.com**.

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