# cador TKP PCR Reagent on the Rotor-Gene® Q

The components of the cador TKP PCR Reagent (cat. no. CD285115) should be stored at -30°C to -15°C and are stable until the expiration date stated on the label. Avoid repeated thawing and freezing (> 2x), as this may reduce sensitivity. Freeze the components in aliquots if they will only be used intermittently. Storage at 2-8°C should not exceed a period of 5 hours.

## Further information and support

- cador TKP PCR Reagent manual: **www.indical.com/handbooks**
- Technical assistance: **support@indical.com**

## Important notes before starting

- Precool the cooling block (Rotor-Gene Q thermal cycler accessory) to 2-8°C.
- Before each use, thaw all reagents completely at room temperature (15-25°C), then mix by pulse-vortexing and centrifuge briefly.
- Place samples and TKP Control DNA in a cooling block at 2-8°C or on ice.
- Include at least one positive control and one no-template control per PCR run.



INDICAL BIOSCIENCE

## Protocol

1. Prepare a master mix according to Table 1. Prepare a volume at least 10% greater than that required for the total number of PCR assays to be performed.

Table 1. Preparation of master mix

| Component | Volume per reaction | Final concentration |
|---|---|---|
| 5x Pathogen Master Mix | 5 µl | 1x |
| 1.66x Primer/Probes | 15 µl | 1x |
| **Total volume** | **20 µl** | **-** |

2. Pipet 20 µl of the master mix into each reaction tube and add 5 µl of the sample DNA.
3. Prepare the positive control (5 µl TKP Control DNA added to 20 µl master mix) and no-template control (5 µl PCR-grade water or QuantiTect Nucleic Acid Dilution Buffer added to 20 µl master mix).
4. Close the reaction tubes with the corresponding caps.
5. Create a temperature profile. Open the “New Run Wizard” of the Rotor-Gene Q software. Check “Locking Ring Attached” and click “Next”.
6. Select “25” for the reaction volume and click “Next”.
7. Click “Edit Profile” and program the temperature profile with the conditions given in Table 2.

Table 2. Cycling protocol

| Step | Temperature | Time | Number of cycles |
|---|---|---|---|
| **Initial Activation** | 95°C | 5 min | 1 |
| **3-step cycling** | | | |
| Denaturation | 95°C | 15 s | |
| Annealing* | 60°C | 45 s | 40 |
| Extension | 72°C | 20 s | |

* Data acquisition performed at this step. Gain optimization before first acquisition.

8. Select the green, yellow, orange, and crimson channels.
9. Click “Gain Optimisation” in the “New Run Wizard” dialog box to open the “Auto-Gain Optimisation Setup” dialog box.
10. Highlight “Acquiring Channels” and click “Add”.
11. Set the calibration temperature to “60”. Check the box “Perform Optimisation before 1st Acquisition”.
12. Click “Start Run” and save the run in a folder of your choice.
13. Analyse the data after the run. Fluorescence signals for bacterial DNA are detected in the green, orange and crimson channels. Signals for the Internal Control are detected in the yellow channel (Table 3).

Table 3. Fluorescence channels

| **DNA/ Internal Control** | **Channel** |
|---|---|
| *Klebsiella pneumoniae* DNA | Green |
| *Pseudomonas aeruginosa* DNA | Orange |
| *Taylorella equigenitalis* DNA | Crimson |
| Internal Control | Yellow |

14. To analyse the run, open the “Analysis” view for each of the channels. Select ”Dynamic Tube” and set the threshold by selecting an appropriate value or by moving the red threshold line up and down using the mouse. The threshold must be adjusted for each of the channels individually.

## Interpretation of results

The following results are possible if working with unknown samples.

**The sample is positive for one or more of the pathogens if there is a signal in the green, orange, and/or crimson channels.**

A fluorescence signal in the green channel indicates the sample is positive for *Klebsiella pneumoniae*. A fluorescence signal in the orange channel indicates the sample is positive for *Pseudomonas aeruginosa*. A fluorescence signal in the crimson channel indicates the sample is positive for *Taylorella equigenitalis*. A fluorescence signal in more than one channel indicates the sample is positive for each of the pathogens detected in that channel.

**The sample is negative for all three pathogens if there is no signal in the green, orange, and crimson channels, but there is a signal in the yellow channel.**

In the case of a negative PCR result for pathogens, a fluorescence signal for the Internal Control rules out the possibility of PCR inhibition.

**The results are inconclusive if there is no fluorescence in any of the channels.**

If no bacterial signal is detected in the green, orange, and crimson channels, and there is no signal for the Internal Control in the yellow channel, the result is inconclusive. The absence of a signal for the Internal Control indicates PCR inhibition or other malfunctions.

Check that there is a fluorescence signal in the green, orange, and crimson channels for the positive control reaction (TKP Control DNA). Absence of a signal for the TKP Control DNA indicates an error in the pathogen DNA amplification, which could be due to incorrect setup of the master mix or incorrect cycling conditions.

## Change index

| Handbook | Version | Change |
|---|---|---|
| HB-2519-EN-002 | Feb 2019 | INDICAL design |