March 2021

Quick-Start Protocol

QIAprep&amp CRISPR Kit

The QIAprep&amp CRISPR Kit (cat. nos. 232101 and 232102) can be used for characterization of CRISPR editing events. It allows rapid processing of cultured cells and fast amplification of a genomic region of interest. The lysate can directly be used for the amplification of the target region without additional purification.

The QIAprep&amp CRISPR Kits should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. After the first thawing, the Cell Lysis Buffer (mat. no. 1123508) should be stored at 2–8°C.

Further information

- *QIAprep&amp CRISPR Handbook*: **www.qiagen.com/HB-2863**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for adherent and suspension-cultured cells derived from human, mouse, and rat. Processing of cells of different origin might require further optimization.
- Spin down the tubes before opening for the first time to collect contents at the bottom of the tube.
- **Add 80 µl Proteinase K to 12 ml CRISPR-Q™ Lysis Buffer, mix well, and store at 2–8°C. Be sure to briefly mix the buffer before each use.**
- The AllTaq™ PCR Master Mix contains AllTaq DNA Polymerase, which is inactive at room temperature (15–25°C). Hence, it is not necessary to keep PCR tubes on ice.
- The Master Mix Tracer is provided as a 125x concentrate and can be directly added to the reaction setup (Table 1) to obtain a 1x final concentration.
  Its use is optional.

- Reactions containing the Master Mix Tracer can be directly loaded onto agarose gels after cycling. The dye runs at 50 bp on a 1% agarose gel.
- When using other amplicon detection methods than agarose gels (e.g., QIAxcel® Advanced), the Master Mix Tracer should not be added to the PCR reaction.

Sample lysis: Adherent cells

The volumes are optimized for confluent adherent cells in 96-well plates. Detailed information on additional plate formats can be found in the handbook. When working with trypsinized cells or semi-adherent cells, collect floating cells and treat them as described in "Sample lysis: Suspension cells".

1. Preheat a thermal cycler at 80°C.
2. Wash the cells with PBS.
3. Lyse the cells by adding 50 µl Cell Lysis Buffer supplemented with Proteinase K.
4. Incubate for 15 min at room temperature.
5. After incubation, pipet the cell lysate 10–15x times up and down and transfer the lysate to an appropriate tube or plate.
6. Incubate the lysate for 10 min at 80°C in a thermal cycler.
7. The cell lysate can directly be used for the PCR amplification or stored at –20°C.

   Note: It is recommended to store the lysate in small aliquots to avoid repeated freeze–thaw cycles.

Sample lysis: Suspension cells

1. Preheat a thermal cycler at 80°C.
2. Harvest CRISPR-edited cells at 500 x *g* for 5 min at 4°C.

   Note: Centrifugation speed and time might need to be adjusted depending on cell type and cell number.
3. Carefully remove the supernatant and wash cells with PBS.

4. Resuspend the cell pellet in Cell Lysis Buffer containing Proteinase K. The volume of the lysis buffer is dependent on the cell number. Optimally, the lysate should contain 10–3000 cells/µl.

   Note: Exceeding the upper limit of cells per microliter can negatively affect lysate preparation and PCR performance.

5. Incubate cell suspension for 15 min at room temperature.
6. After incubation, pipet cell lysate 10–15x times up and down.
7. Continue with step 5 of the "Sample lysis: Adherent cells" protocol.

PCR amplification of target region using CRISPR-Q Custom PCR Assays

1. Thaw all PCR components listed in Table 1 and mix thoroughly before use.
2. Prepare a reaction mix according to Table 1.

   Note: A negative control (without template) and a positive control using the provided CRISPR-Q Control PCR Assay should be included in every experiment.

Table 1. PCR reaction setup

Component	Volume/reaction	Final concentration
AllTaq Master Mix, 4x	5 µl	1x
CRISPR-Q Custom PCR Assay, 20x	1 µl*	1x
Cell lysate (template)	5 µl	10–40,000 cells/reaction†
Optional: Master Mix Tracer, 125x	0.16 µl	1x
Optional: Q-Solution®, 5x	4 µl‡	1x
RNase-free water	Variable	-
Total reaction volume	**20 µl**	

* When using CRISPR-Q Control PCR Assay (20x), please add 1 µl per reaction. When using other assays, please refer to the *AllTaq PCR Core and Master Mix Kits Handbook* for more information.

† Number of cells per reaction is dependent on lysis protocol, cell type, plate format, and target of choice. Cell number may need further optimization. See handbook for more information.

‡ The QIAprep&amp CRISPR Kit is provided with Q-Solution, which facilitates amplification of templates that have a high degree of secondary structure or that are GC-rich. When using Q-Solution for the first time with a particular primer-template system, always perform parallel reactions with and without Q-Solution.

3. Mix the reaction mix gently but thoroughly and dispense appropriate volumes into PCR tubes or wells of a PCR plate.

4. Place the PCR tubes or plates in the thermal cycler and start the PCR program as outlined in Table 2.

   Note: After amplification, samples can be stored at –20°C for longer storage.

Table 2. PCR cycling conditions*

Step	Time	Temperature	Comment
Initial PCR activation	3 min	95°C	This heating step activates AllTaq DNA Polymerase
3-step cycling			
Denaturation	30 s	95°C	
Annealing	30 s	60°C	Temperature might differ dependent on the T_m of primers
Extension	45 s	72°C	
Number of cycles	40		
Final extension	5 min	72°C	

* Cycling conditions are optimized for CRISPR-Q Custom PCR Assays and the CRISPR-Q Control PCR Assay.

Document Revision History

Date	Changes
03/2021	Initial release

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.