Quick-Start Protocol October 2015

miScript® Single Cell qPCR Kit

Protocols: Cell Lysis, 3′ Ligation, 5′ Ligation, Reverse-Transcription

Further information

- *miScript Single Cell qPCR System Handbook*: **www.qiagen.com/handbooks**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000, or **www.qiagen.com/contact**

Notes before starting

- Add 2.5 µl Buffer FCPM to Buffer FCPL, mix well and store at 2–8°C. This only needs to be added once. Briefly shake or vortex the FCPL / FCPM mixture before each use.
- Add 750 µl RNase-free water to lyophilized gDNA Wipeout Buffer 2. Mix by gently inverting the vial, divide into single-use aliquots and store at –20°C.
- Prepare the reagents required for each protocol according to the *miScript Single Cell qPCR System Handbook*.
- Use 1–100 cells as starting material. The cells should be in 2 µl or less of neutral buffer like PBS and thawed on ice.
- The 3′ ligation and 5′ ligation reactions are very viscous. Pipet slowly and thoroughly (pipet up and down 12 times) to mix.
- Do not vortex reactions or reagents unless instructed.
- Ensure master mixes are centrifuged briefly before mixing, thoroughly mixed by pipetting up and down 12 times and briefly centrifuged once again.
- Ensure reactions are thoroughly mixed, prepared at recommended temperatures and incubated at recommended temperatures.

Cell Lysis

1. Prepare the cell processing master mix on ice according to Table 1.

Table 1. Preparation of cell processing master mix

Component	Master mix 24 samples	Master mix 96 samples
Buffer FCPL/FCPM	124 µl	496 µl
gDNA Wipeout Buffer	8 µl	32 µl
Total volume	**132 µl**	**528 µl**

2. Add 3 µl of cell processing master mix into each well of the PCR strip tubes or 96-well plates containing a cell in 2 µl PBS. Do not mix the cell / cell processing master mix.

   Important: If the cells are in less PBS, bring final volume to 5 µl with additional mix.

3. Incubate for 5 min at room temperature, 5 min at 75°C and hold at 4°C.
4. Proceed to *3′ Ligation*. Alternatively, the lysed cells can be stored at –80°C.

3′ Ligation

1. Thaw PCR strip tube(s) or 96-well plate containing cell lysate on ice. Centrifuge briefly.
2. Prepare the 3′ ligation reaction master mix at room temperature according to Table 2.

Table 2. Setup of 3′ ligation reactions

Component	Volume one sample	Master mix 24 samples	Master mix 96 samples
Cell lysate (already in the well of a PCR strip tube or 96-well plate)	5 µl	–	–
miScript SC 3′ Ligation Buffer	1 µl	27 µl	108 µl
Nuclease-free water	0.5 µl	13.5 µl	54 µl
miScript SC 3′ RNA Ligase	0.5 µl	13.5 µl	54 µl
miScript SC Ligation Activator (Step 4)	8 µl	–	–
Total volume	**15 µl**	**54 µl**	**216 µl**

3. At room temperature, aliquot 2 µl of the 3′ ligation master mix into each well of the PCR strip tubes or 96-well plates containing 5 µl cell lysate.
4. Slowly add miScript SC Ligation Activator to each well. Briefly centrifuge, mix by pipetting up and down 12 times and briefly centrifuge again.

   Important: Pipet slowly to mix. miScript SC Ligation Activator is very viscous.
5. Incubate for 1 h at 16°C and hold at 4°C.
6. Proceed immediately to *5′ Ligation*.

5′ Ligation

1. Immediately prior to setting up the 5′ ligation reactions, allow the completed 3′ ligation reactions to equilibrate to room temperature for 2 min. Centrifuge briefly.
2. Prepare the 5′ ligation reaction master mix at room temperature according to Table 3.

Table 3. Setup of 5′ ligation reactions

Component	Volume one sample	Master mix 24 samples	Master mix 96 samples
3′ ligation reaction (already in the well of a PCR strip tube or 96-well plate)	15 µl	–	–
miScript SC 5′ Ligation Buffer	4 µl	108 µl	432 µl
miScript SC 5′ RNA Ligase	1 µl	27 µl	108 µl
Total volume	**20 µl**	**135 µl**	**540 µl**

3. At room temperature, aliquot 5 µl of the 5′ ligation master mix into each well of the PCR strip tubes or 96-well plates containing the completed 3′ ligation reaction. Briefly centrifuge, mix by pipetting up and down 12 times and briefly centrifuge again.

   Important: Pipet slowly to mix. The reactions are very viscous.
4. Incubate for 5 min at 37°C, 15 min at 65°C and hold at 4°C.

   Important: Hold at 4°C for at least 5 min.

5. Proceed immediately to *Reverse-Transcription*.

Reverse-Transcription

1. Prepare the reverse-transcription reaction master mix on ice according to Table 4.

Table 4. Setup of reverse-transcription reactions

Component	Volume one sample	Master mix 24 samples	Master mix 96 samples
5′ ligation reaction (already in the well of a PCR strip tube or 96-well plate)	20 µl	–	–
5x miScript SC RT Buffer	8 µl	216 µl	864 µl
10x miScript SC RT Nucleics	4 µl	108 µl	432 µl
Nuclease-free water	7 µl	189 µl	756 µl
miScript SC Reverse Transcriptase	1 µl	27 µl	108 µl
Total volume	**40 µl**	**540 µl**	**2160 µl**

2. On ice, aliquot 20 µl of the reverse-transcription master mix into each well of the PCR strip tubes or 96-well plates containing the completed 5′ ligation reaction.
3. Incubate for 2 h at 37°C, 5 min at 95°C and hold at 4°C.

   Important: Hold at 4°C for at least 5 min.
4. Proceed to the second Quick-Start protocol associated with the miScript Single Cell qPCR Kit (Protocols: "Beads + Bind" Mixture Preparation, cDNA Cleanup, Preamplification). Alternatively, the completed cDNA syntheses can be stored at –20°C in a constant-temperature freezer.