Quick-Start Protocol October 2015

miScript® Single Cell qPCR Kit

Protocols: "Beads + Bind" Mixture Preparation, cDNA Cleanup, Preamplification

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

- Prepare the reagents required for each protocol according to the *miScript Single Cell qPCR System Handbook*.
- miScript SC Cleanup Beads need to be thoroughly mixed and homogeneous. This necessitates working quickly and vortexing the beads immediately before use. If a delay in the protocol occurs, simply re-vortex the beads.
- Ensure the miScript SC Cleanup Bind, "Beads + Bind" mixture and the synthesized cDNA are ice cold at all times.
- Do not vortex the 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.

"Beads + Bind" Mixture Preparation

1. Determine how many "Beads + Bind" mixtures need to be prepared and repeat steps 2–7 for each mixture. Each "Beads + Bind" mixture provides enough mixture for the cleanup of eight cDNA syntheses.
2. Thoroughly resuspend the miScript SC Cleanup Beads by briefly vortexing.
3. Pipet 13.5 µl of the miScript SC Cleanup Beads into a microcentrifuge tube.
4. Pipet 100 µl of ice cold miScript SC Cleanup Bind onto the beads. Vortex to resuspend.
5. Immediately separate the beads from suspension on a magnetic stand.
6. Using a pipet, remove the supernatant while taking care not to disturb the beads.
7. Pipet 360 µl of ice cold miScript SC Cleanup Bind onto the beads. Vortex to resuspend.
8. The "Beads + Bind" mixture for the cleanup of eight cDNA syntheses is now prepared and must be stored on ice.
9. Proceed immediately to *cDNA Cleanup*.

cDNA Cleanup

1. Ensure the "Beads + Bind" mixture is thoroughly mixed at all times.
2. Add 40 µl of the "Beads + Bind" mixture to each cDNA and vortex.
3. Using an Eppendorf® MixMate®, shake at 1100 rpm for 10 min.
4. Separate the beads from suspension on a magnetic stand. Discard supernatant.
5. Add 150 µl of freshly prepared 80% ethanol to the beads. Mix by vortexing.
6. Separate the beads from suspension on a magnetic stand. Discard supernatant.
7. Repeat steps 5–6.
8. Ensure all residual ethanol has been removed, and air dry the beads for 10 min.
9. Add 20 µl Buffer EB to the beads. Mix by vortexing.
10. Using an Eppendorf MixMate, shake at 1100 rpm for 5 min.
11. Separate the beads from suspension on a magnetic stand.

12. Pipet 16 µl from each well into a new well of a PCR strip tube or 96-well plate.
13. Proceed to *Preamplification*. Alternatively, the cleanup products can be stored at –20°C in a constant-temperature freezer.

Preamplification

1. Thaw PCR strip tube(s) or 96-well plate containing the cleanup product. Centrifuge briefly.
2. Prepare the preamplification reaction master mix on ice according to Table 1.

Table 1. Setup of preamplification reactions

Component	Volume one sample	Master mix 24 samples	Master mix 96 samples
Cleanup product (already in the well of a PCR strip tube or 96-well plate)	16 µl	–	–
miScript SC PreAMP Buffer	5 µl	135 µl	540 µl
miScript SC PreAMP Universal Primer	2 µl	54 µl	216 µl
HotStarTaq DNA Polymerase	2 µl	54 µl	216 µl
Total volume	**25 µl**	**243 µl**	**972 µl**

3. On ice, aliquot 9 µl of the preamplification master mix into each well of the PCR strip tubes or 96-well plates containing the cleanup product. Briefly centrifuge, mix by pipetting up and down 12 times and briefly centrifuge again.
4. Program the thermal cycler according to Table 2.

   Important: The basic protocol in Table 2 (12 total cycles) should be used when qPCR will be performed on plate-based cyclers / Rotor-Discs® when 1–8, 96-well plates or 1–2, 384-well plates will be used. For additional formats, please refer to Table 2 footnotes.

Table 2. Preamplification

Step	Time	Temperature
PCR initial activation step	15 min	95°C
3-step cycling		
Denaturation	30 s	94°C
Annealing	60 s	55°C
Extension	60 s	70°C
Cycle number	2 cycles	
2-step cycling		
Denaturation	30 s	94°C
Annealing/extension	3 min	60°C
Cycle number*†	10 cycles*†	
Hold at 4°C	∞‡	

* Adjust to 11 cycles when qPCR will be performed on the Fluidigm® BioMark™ HD or on plate-based cyclers/Rotor-Discs when 9–16, 96-well plates or 3–4, 384-well plates will be used.

† Adjust to 12 cycles when qPCR will be performed on plate-based cyclers / Rotor-Discs when 17–32, 96-well plates or 5–8, 384-well plates will be used.

‡ Hold at 4°C for at least 5 min.

5. Place the preamplification reaction in the thermal cycler and start the run.
6. Immediately upon completion of the preamplification cycling, add 2 µl Side Reaction Reducer to each reaction. Thoroughly mix by pipetting and avoiding excess bubbles.
7. Incubate for 30 min at 37°C, 5 min at 95°C and hold at 4°C.
8. Dilute preamplified product to 127 µl using 100 µl nuclease-free water.
9. Proceed to *Protocol: Real-time PCR Quality Control* in the *miScript Single Cell qPCR System Handbook*. Alternatively, the diluted reactions can be stored at –20°C in a constant-temperature freezer.

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