Protocol: XENO-Q miRNA Detection Kit

Part A. XENO-Q Reaction

1. Prepare XENO-Q reaction as indicated in Table 1. Mix thoroughly with a vortex and centrifuge reaction tubes briefly to spin down the contents and eliminate air bubbles.

Table 1. Set-up for XENO-Q Reaction

Component	Volume per reaction, µl
RNA	x
Nuclease-free distilled water	10-x
Xeno-pol reaction premix	10
Total volume	20

2. Incubate the complete reaction mix according to the guidelines in Table 2 using a thermal cycler with the heated lid on.

Table 2. Reaction conditions of XENO-Q Reaction

Temperature (°C)	Incubation time (min)
95	0.5
63	10

3. After the reaction, transfer the samples directly to the ice rack.

Part B. Nuclease Treatment

4. Prepare the nuclease reaction mix as indicated in Table 3. Briefly mix with a vortex, to thoroughly mix the contents. Centrifuge the reaction tubes briefly to spin down the contents and eliminate air bubbles.

Table 3. Set-up for Nuclease Reaction Mixture

Component	Volume per reaction, µl
Xeno-nuclease mixture	2
Xeno-nuc buffer	18
Total volume	20

5. Mix XENO-Q reaction sample (20 µl) and nuclease reaction mixture (20 µl) thoroughly by vortexing. Centrifuge the reaction tubes briefly to spin down the contents and eliminate air bubbles.
6. Place the reaction tubes into a thermal cycler, then incubate at 60°C for 10 min.
7. After the reaction, transfer the samples directly to the ice rack.

Part C. Column Elution

8. Add 100 µl of column buffer to the reaction tubes, vortex briefly to thoroughly mix the contents, and then centrifuge the reaction tubes briefly to spin down the contents.
9. Place XENO-Q™ clean-up column in a new 1.5 ml collection tube and pipet up to 200 µl of the mixture into a column. Close the lid and centrifuge at 2500 x *g* for 1 min at 25°C.
10. Discard the column and vortex briefly to thoroughly mix the contents, and then centrifuge the reaction tubes briefly to spin down the contents.
11. Proceed to performing the real-time PCR (real-time PCR section)

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Part D. Real-Time PCR

1. In a 1.5-mL microcentrifuge tube, prepare sufficient amount of PCR Reaction Mix for the required number of reactions according to the Table 4 shown below.

Table 4. Real-time PCR Reaction mix

Component	Volume per reaction, µl
XENO QPCR premix	10
Probe/primer mix	5
Total volume	15

Table 5. Probe/primer mix (human miRNA detection kit)

Probe/primer mix	Target miRNA	Fluorescence
hsa-miR-1260b probe	*hsa-miR-1260b*	FAM
hsa-miR-423-5p probe	*hsa-miR-423-5p*	FAM
hsa-miR-378a-3p probe	*hsa-miR-378a-3p*	FAM

Table 6. Probe/primer mix (mouse miRNA detection kit)

Probe/primer mix	Target miRNA	Fluorescence
mmu-miR-423-5p probe	*mmu-miR-423-5p*	FAM
mmu-miR-378a-3p probe	*mmu-miR-378a-3p*	FAM

2. Vortex PCR Reaction Mix to thoroughly mix the contents, then centrifuge briefly to spin down the contents.
3. Add 5 µl of cDNA template to each reaction well of the plate.
4. Transfer 15 µl of PCR Reaction Mix to each well of PCR reaction plate. The total volume should be 20 µl per reaction well.
5. Seal the reaction plate with an adhesive cover, then vortex briefly to thoroughly mix the contents.
6. Centrifuge the reaction plate briefly to spin down the contents.
7. Load the reaction plate in the real-time PCR instrument.
8. Set an appropriate experiment setting and PCR thermal cycling conditions for your instrument.

Table 7. PCR conditions

Step	Temperature	Time	Cycles
Enzyme activation	95°C	5 minutes	1
Denaturation	95°C	10 seconds	40
Annealing / Extension	60°C	30 seconds	

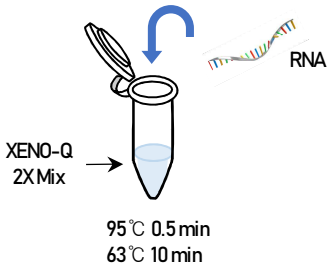
9. Set the reaction volume appropriate for the reaction plate.
10. Start the run.

Quick Guide

Protocol : XENO-Q

Part A. XENO-Q Reaction

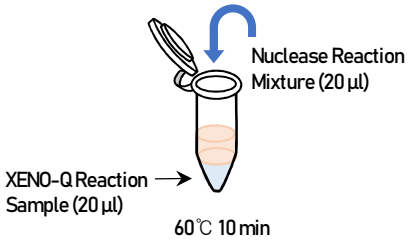
1. Mix the RNA with XENO-Q 2X Mix and incubate at 95℃ for 0.5 min and 63℃ for 10 min using a PCR cycler.



2. After the reaction, transfer the samples directly to the ice rack.

Part B. Nuclease Treatment

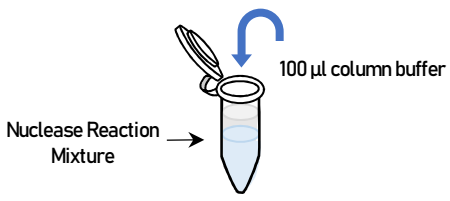
3. Add 20 µl of nuclease reaction mixture and mix thoroughly by vortexing. Incubate the prepared sample at 60℃ for 10 min using a PCR cycler.



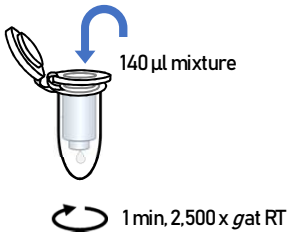
4. After the reaction, transfer the samples directly to the ice rack.

Part C. Column Elution

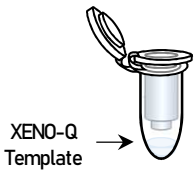
5. Add 100 µl of column buffer to the reaction tubes, vortex briefly to thoroughly mix the contents, and then centrifuge the reaction tubes briefly to spin down the contents.



6. Place XENO-Q™ clean-up column in a new 1.5 ml collection tube and pipet up to 140 µl of the mixture into a column. Close the lid and centrifuge at 2,500x*g* for 1 min at RT.



7. Discard the column and vortex briefly to thoroughly mix the contents, and then centrifuge the reaction tubes briefly to spin down the contents.



Part D. Real-Time PCR

8. Proceed to performing the real-time PCR.

Kit Contents (Human Kit)

Number	Kit contents	Amount	Quantity
1	XENO-Q 2X Mix	10 µl	48
2	Nuclease Mix	100 µl	1
3	Nuclease Buffer	900 µl	1
4	Column Buffer	1600 µl	5
5	2x XENO qPCR premix	1000 µl	5
6	hsa-miR-1260b probe	375 µl	2
7	hsa-miR-423-5p probe	375 µl	2
8	hsa-miR-378a-3p probe	375 µl	2
9	Clean-up Column	-	48

Kit Contents (Mouse Kit)

Number	Kit contents	Amount	Quantity
1	XENO-Q 2X Mix	10 µl	48
2	Nuclease Mix	100 µl	1
3	Nuclease Buffer	900 µl	1
4	Column Buffer	1600 µl	5
5	2x XENO qPCR premix	1000 µl	3
6	mmu-miR-423-5p probe	375 µl	2
7	mmu-miR-378a-3p probe	375 µl	2
8	Clean-up Column	-	48