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Single Cell-to-CT™ Kit

Catalog Numbers 4458236 and 4458237

Pub. no. 4458354 **Rev**. E

Component	Quantity		Storage conditions
	50 rxns (Cat. no. 4458237)	400 rxns (Cat. no. 4458236)	
Single Cell DNase I	50 µL	400 µL	–20°C
Single Cell Lysis Solution	0.5 mL	4.0 mL	4°C
Single Cell PreAmp Mix	265 µL	2.1 mL	–20°C
Single Cell Stop Solution	50 µL	400 µL	–20°C
Single Cell SuperScript® RT	75 µL	600 µL	–20°C
Single Cell VILO™ RT Mix	150 µL	1.2 mL	–20°C
TaqMan® Gene Expression Master Mix	5.0 mL	50 mL	4°C

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Single Cell-to-CT™ Kit User Guide* (Pub. no. 4458356). For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This insert provides abbreviated instructions for using the Single Cell-to-CT™ Kit. For detailed instructions and troubleshooting information, refer to the *Single Cell-to-CT™ Kit User Guide* (Pub. no. 4458356). You can download a PDF version of the protocol from the Life Technologies web site at **www.lifetechnologies.com**.

Perform single-cell lysis

1. Add 1 µL Single Cell DNase I to 9 µL Single Cell Lysis Solution.
2. Add 1–10 cells to the 10 µL of Single Cell DNase I/Single Cell Lysis Solution (no mixing required), then incubate at room temperature for 5 minutes.
3. Add 1 µL Single Cell Stop Solution (no mixing required), incubate at room temperature for 2 minutes, then place the sample on ice.

Perform reverse transcription

1. Prepare sufficient RT master mix for all samples, then add 4.5 µL to each lysed cell sample:

Component	Volume for each reaction
Single Cell VILO™ RT Mix	3 µL
Single Cell SuperScript® RT	1.5 µL
Total volume for each reaction	**4.5 µL**

2. Perform reverse transcription in a thermal cycler:

Temp	Time
25°C	10 min
42°C	60 min
85°C	5 min

Perform preamplification

1. Pool the TaqMan® Gene Expression Assays for your targets of interest, then dilute the pooled assays using 1× TE Buffer, pH 8.0 so that each assay is at a final concentration of 0.2×.
2. Prepare sufficient PreAmp reaction mix, then add 11 µL to each reverse-transcribed sample:

Component	Volume for each reaction
Single Cell PreAmp Mix	5 µL
0.2× pooled TaqMan® Gene Expression Assays	6 µL
Total PreAmp reaction mix	**11 µL**

3. Perform preamplification in a thermal cycler:

Stage	Step	Temp	Time
Holding	Enzyme activation	95°C	10 min
Cycling (14 cycles)	Denature	95°C	15 sec
	Anneal/extend	60°C	4 min
Holding	Enzyme deactivation	99°C	10 min

4. Place the tubes on ice or store at –20°C.

Real-time PCR recommendations

Reaction conditions

Note: Dilute the preamplified products with 1X TE Buffer, pH 8.0 before performing real-time PCR.

- Using 96-well or 384-well reaction plates:

Component	Volume for each reaction	
	96-well plate	384-well plate
2X TaqMan® Gene Expression Master Mix	25.0 µL	10.0 µL
Preamplified product diluted 1:20 with 1X TE Buffer, pH 8.0	10.0 µL	4.0 µL
20X TaqMan® Gene Expression Assay	2.5 µL	1.0 µL
Nuclease-free water	12.5 µL	5.0 µL
Total volume for each reaction	**50 µL**	**20 µL**

- Using a TaqMan® Array:

Component	Volume for a full array
2X TaqMan® Gene Expression Master Mix	450 µL
Preamplified product diluted 1:40 with 1X TE Buffer, pH 8.0	450 µL
Total volume for the array	**900 µL**

Real-time PCR conditions

Stage	Step	Temp	Time
Holding	UDG incubation	50°C	2 min
Holding	Enzyme activation	95°C	10 min
Cycling (40 cycles)	Denature	95°C	5 sec
	Anneal/extend	60°C	1 min

Analysis conditions

- Use an automatic baseline and set the threshold to 0.2.
- Review the amplification plots and remove outliers.
- Omit samples that are undetectable for all assays tested.
- Use RealTime StatMiner® Software to perform differential expression analysis.

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