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Stabilized Blood-to-C_T™ Nucleic Acid Preparation Kit for qPCR

(Compatible with PAXgene® Blood RNA Tubes)

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Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Stabilized Blood-to-C_T™ Nucleic Acid Preparation Kit for qPCR User Guide* (Part No. 4449675). For every chemical, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference card includes step-by-step instructions for preparation of reverse transcription-PCR-ready lysates from PAXgene® Blood RNA Tube-stabilized blood samples using the Stabilized Blood-to-C_T™ Nucleic Acid Preparation Kit for qPCR (Part no. 4449082), and guidelines for reverse transcription and real-time PCR with recommended reagents. For background information, troubleshooting, and supplemental procedures, refer to the *Stabilized Blood-to-C_T™ Nucleic Acid Preparation Kit for qPCR User Guide*.

Pellet and wash the sample

Pellet the sample

1. If the PAXgene® Blood RNA Tubes have been frozen, allow them to thaw at room temperature for at least 2 hours.
2. Transfer 500 µL of PAXgene stabilized blood to:
   - A 1.5-mL microfuge tube

     OR
   - MagMAX™ Express-96 Deep Well Plate (also referred to as *Express-96 plate*)

   IMPORTANT! You must use a MagMAX™ Express-96 Deep Well Plate. This protocol has not been optimized for use with any other plate.
3. Centrifuge the tube or plate at 5000 × *g* for 10 minutes to pellet the sample.
4. With a pipette, remove and discard the supernatant, being careful not to disturb the pellet. Some residual supernatant may be left behind (up to 50 µL).

Wash the sample

1. Add 750 µL of Wash Solution (Compatible with PAXgene® Blood RNA Tubes) to the tube or plate, then cap the tube or seal the plate firmly with a MicroAmp® adhesive film.

   IMPORTANT! Make sure that the Express-96 plate is sealed securely; if the wells are not sealed firmly, cross-contamination of samples may occur.
2. Vortex the tube or plate until the pellet is dissolved.

   Note: The pellet may resuspend in Wash Solution before vortexing. If the pellet is large and does not resuspend easily, vortex for 2 minutes, then proceed to step 3.

   STOPPING POINT You can store the mixture overnight at –20°C.
3. To pellet the sample, centrifuge the:
   - Tube at 5000 × *g* for 2 minutes
   - Plate at 5000 × *g* for 5 minutes
4. With a pipette, remove and discard the Wash Solution, being careful not to disturb the pellet. Some residual wash may be left behind (up to 50 µL).
5. Add 750 µL of Wash Solution to the tube or plate, then cap the tube or seal the plate firmly with a MicroAmp® adhesive film.
6. Vortex until the pellet is dissolved.

   Note: The pellet may resuspend in Wash Solution before vortexing. If the pellet is large and does not resuspend easily, vortex for 2 minutes, then proceed to step 7.
7. To pellet the sample, centrifuge the:
   - Tube at 5000 × *g* for 2 minutes
   - Plate at 5000 × *g* for 5 minutes
8. With a pipette, completely remove and discard Wash Solution, being careful not to disturb the pellet.
9. Place the tube or plate on ice or hold at 4°C.

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Perform digestion

Thaw the Stop Solution

1. Thaw the Stop Solution at room temperature
2. After thawing, mix by flicking or inverting the tube several times, then place the tube on ice.

(Optional) Mix the Digestion Solution with DNase I

To remove genomic DNA during digestion, dilute the DNase I into the Digestion Solution at 1:100.

1. Per the table below, calculate the total volume required for each component: *volume for 1 reaction × the total number of reactions*

   Include excess volume in your calculations to compensate for the loss that occurs during pipetting.

Component	Vol. for 1 reaction
Digestion Solution	99 µL
DNase 1	1 µL
Total volume	**100 µL**

2. Add the components to a microcentrifuge tube, then mix gently by pipetting up and down several times.

Add the Digestion Solution and incubate

1. Add 100 µL of room-temperature Digestion Solution (with or without DNase 1) to the microfuge tube or Express-96 plate (containing the pellet).
2. Mix by pipetting up and down 5 times to break up the pellet. To avoid bubbles, mix with the pipette set at 70 µL and do not completely empty the pipette tip.
3. Incubate at room temperature (19 to 25°C) for 8 minutes.

   Note: The Digestion Solution and samples may appear cloudy at room temperature; this is expected.

(Optional) Mix the Stop Solution with Xeno™ RNA Control

To include an endogenous control using the TaqMan® Cells-to-C_T Control Kit, add Xeno™ RNA Control to the Stop Solution.

1. Per the table below, calculate the total volume required for each component: *volume for 1 reaction × the total number of reactions*

   Include excess volume in your calculations to compensate for the loss that occurs during pipetting.

Component	Vol. for 1 reaction
Stop Solution	10 µL
Xeno™ RNA Control	2 µL
Total volume	**12 µL**

2. Add the components to a microcentrifuge tube, then mix gently by pipetting up and down several times.
3. Place the mixture on ice.

Add the Stop Solution and incubate

1. Add 10 µL of the Stop Solution, or 12 µL of the Stop Solution/Xeno RNA Control mixture, to the microfuge tube or Express-96 plate (containing the sample).

   Touch the surface of the liquid sample with the opening of the pipette tip to ensure that all of the Stop Solution is added to the sample.
2. Mix by pipetting up and down 5 times. To avoid bubbles, mix with the pipette set at 70 µL and do not completely empty the pipette tip.

   IMPORTANT! Be sure to thoroughly mix the Stop Solution into the sample.
3. Incubate at room temperature (19 to 25°C) for 2 minutes.

   Note: After adding the Stop Solution, do not allow the samples to remain at room temperature for >20 minutes.

STOPPING POINT You can store the samples at 4°C for up to 1 hour, or at –20 to – 80°C for up to 5 months.

Perform qRT-PCR

This section provides guidelines for using the prepared samples in reverse transcription and real-time PCR with recommended RT-PCR reagent combinations.

High Capacity RNA-to-cDNA™ Kit and TaqMan® Gene Expression Master Mix

Component	Vol. for 1 20-µL reaction
RT master mix	
2X RT Buffer	10 µL
20X RT Enzyme Mix	1 µL
Nuclease-free water	5 µL
Sample	4 µL
Total volume of RT master mix	**20 µL**
PCR mix	
TaqMan® Gene Expression Master Mix (2X)	10 µL
TaqMan® Gene Expression Assay (20X)	1 µL
Nuclease-free water	5 µL
cDNA	4 µL
Total volume of PCR mix	**20 µL**

SuperScript® VILO™ cDNA Synthesis Kit and TaqMan® Gene Expression Master Mix

Component	Vol. for 1 20-µL reaction
RT master mix	
5X VILO™ Reaction Mix	4 µL
10X SuperScript® Enzyme Mix	2 µL
Nuclease-free water	10 µL
Sample	4 µL
Total volume RT master mix	**20 µL**
PCR mix	
TaqMan® Gene Expression Master Mix (2X)	10 µL
TaqMan® Gene Expression Assay (20X)	1 µL
Nuclease-free water	7 µL
cDNA	2 µL
Total volume of PCR mix	**20 µL**

TaqMan® MicroRNA Reverse Transcription Kit and TaqMan® Universal Master Mix II

Component	Vol. for 1 7.5-µL reaction
RT master mix	
10X RT Buffer	0.8 µL
dNTPs with dTTP (100 mM)	0.2 µL
$MgCl_2$ (25 mM)	0.9 µL
RNase Inhibitor (20 U/µL)	0.1 µL
MultiScribe™ Reverse Transcriptase (50 U/µL)	1.5 µL
RT Primers (10X)	0.8 µL
Nuclease-free water	0.2 µL
Sample	3.0 µL
Total volume RT master mix	**7.5 µL**
PCR mix	
TaqMan® Universal Master Mix II, with or without UNG (2X)	10 µL
TaqMan® Assay (20X)	1 µL
cDNA + nuclease-free water†	9 µL
Total volume of PCR mix	**20 µL**

† To each reaction, add 1 to 100 ng of cDNA diluted to the correct volume using nuclease-free water.

TaqMan® One-Step RT-PCR Master Mix Reagents Kit

Component	Vol. for 1 20-µL reaction
TaqMan® RT Enzyme Mix (40X)	0.5 µL
TaqMan® RT-PCR Mix (2X)	10.0 µL
TaqMan® Gene Expression Assay (20X)	1.0 µL
Nuclease-free water	7.5 µL
Sample	1.0 µL
Total volume of RT-PCR master mix	**20.0 µL**

Kit contents and storage conditions

Kit part number	Kit name	Kit contents			
		Box	Component	Amount	Storage conditions
4449082	Stabilized Blood-to-C_T™ Nucleic Acid Preparation Kit for qPCR, 200 reactions (Compatible with PAXgene® Blood RNA Tubes)	1	Digestion Solution	20 mL	4°C
			Wash Solution (Compatible with PAXgene® Blood RNA Tubes)	300 mL	
		2	Stop Solution	2 mL	–20°C
			DNase 1	200 µL	
4449079†	Stabilized Blood-to-C_T™ Nucleic Acid Preparation Kit for qPCR (50 reactions)	1	Digestion Solution	5 mL	4°C
			Tempus® Pellet Enhancer	1 mL	
			Tempus® Wash #1	37.5 mL	
			Tempus® Wash #2	37.5 mL	
			2× PBS for Tempus® Blood Tubes	12.5 mL	
			Wash Solution (Compatible with PAXgene® Blood RNA Tubes)	75 mL	
		2	Stop Solution	0.5 mL	–20°C
			DNase 1	50 µL	

† Kit part number 4449079 is a starter kit that contains reagents for both the Tempus® and PAXgene® Blood RNA Tubes.

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