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

(Compatible with Tempus® Blood RNA Tubes)

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Stabilized Blood-to-C_T™ Nucleic Acid Preparation Kit for qPCR Protocol* (PN 4449676). 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 covers:

Pellet and wash the sample

Pellet the sample

1. If the Tempus® Blood RNA Tubes have been frozen, allow them to thaw at room temperature (~15 minutes).

2. Transfer 500 µL of Tempus 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. Add 250 µL of 2X PBS for Tempus® 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.

4. Vortex the tube or plate for 10 seconds, then centrifuge briefly to remove droplets from inside of the cap or adhesive film.

5. Add 20 µL of Tempus® Pellet Enhancer to the tube or plate, then cap the tube or seal the plate firmly with a MicroAmp adhesive film.

6. Vortex the tube or plate for 10 seconds, then centrifuge at 5000 × *g* for 10 minutes to pellet the sample.

7. 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 Tempus® Wash #1 to the tube or plate, then cap the tube or seal the plate firmly with a MicroAmp adhesive film.

2. Vortex the tube or plate until the pellet is dissolved.

   Note: The pellet may resuspend in Tempus Wash #1 immediately before vortexing. If the pellet is large and does not resuspend easily, vortex for 2 minutes, then proceed to step 3.

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 Tempus Wash #1, being careful not to disturb the pellet. Some residual supernatant will be left behind (~20 µL).

5. Add 750 µL of Tempus® Wash #2 to the tube or plate, then cap the tube or seal the plate firmly with a MicroAmp adhesive film.

6. Vortex the tube or plate until the pellet is dissolved.

   Note: The pellet may resuspend in Tempus Wash #2 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 Tempus Wash #2, being careful not to disturb the pellet.

9. Place the tube or plate on ice.

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	Volume 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	Volume 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 longer than 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 the reverse transcription and PCR procedures of a quantitation experiment.

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

Two Stabilized Blood-to-C_T kits are available for the Tempus tubes. The package that Applied Biosystems ships each kit in contains two boxes; the boxes contain the components listed in the table below. Upon receipt, open the boxes, then store each component as indicated below.

Kit part number	Kit name	Kit contents			
		Box	Component	Quantity	Storage conditions
4449080	Stabilized Blood-to-C_T™ Nucleic Acid Preparation Kit for qPCR, 200 reactions (Compatible with Tempus® Blood RNA Tubes)	1	Digestion Solution	20 mL	4 °C
			Tempus® Pellet Enhancer	4 mL	
			Tempus® Wash #1	150 mL	
			Tempus® Wash #2	150 mL	
			2× PBS for Tempus®	50 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®	12.5 mL	
			PAXgene® Wash	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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