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Product Data Sheet
Blood RNA Extraction-Magnetic Kit

Cat. #: BREM-100

Product Description:

The Blood RNA Extraction-Magnetic Kit enables scalable and rapid extraction of RNA from whole blood samples. The kit uses magnetic bead technology for the recovery of RNA in elution buffer suitable for various downstream applications. If desired TE Buffer can be added following elution, in place of Elution Buffer for dilution of yielded RNA product.

Kit Contents and Storage:

Reagent quantities provided in each kit are sufficient for 100 reactions. All reagents should be stored in the appropriate conditions upon receipt. Product is shipped at ambient temperature.

Component	Catalog No.	Volume	Quantity	Storage
RNA Blood Lysis and Binding Buffer	R-BLBB-70	70 ml	1	15°C to 30°C
DNA/RNA Binding Beads	DRBB-50	57.5 ml	1	2°C to 8°C
Wash Buffer 1	W1-50	50 ml	1	15°C to 30°C
Elution Buffer	EB-15	15 ml	2	15°C to 30°C
DNASE I	DNASE-100	100 µl	1	-25°C to -15°C
DNASE Buffer	DB-7	7.5 ml	1	15°C to 30°C

Reagent Preparation:

The following mixtures may be prepared prior to performing the extraction procedure:

- 80% Absolute Ethanol
- DNASE Solution (see following table)

Component	Volume per rxn
DNASE I	1 µl
DNASE Buffer	74 µl
Total DNASE Solution	**75 µl**

General Guidelines:

- Perform all procedural steps at room temperature (15°C to 30°C) unless otherwise specified.
- When mixing samples by pipetting up and down, avoid creating bubbles.

Additional Notes:

Precipitation may occur if certain reagents are stored below indicated storage temperature. We recommend warming the precipitated solutions to 37°C for 15 minutes to eliminate the precipitate.

RNA Extraction Procedure:

1. Blood Fractionation, Sample Preparation
   1.1. Centrifuge whole blood sample in order to separate blood samples into its components.
   1.2. Up to **200 µl** of **plasma**, **buffy coat**, or **red blood cells** may be used per reaction.
   1.3. The following reagent volumes are based on **200 µl** sample starting volumes. If smaller volumes of samples are being processed, adjust the following reagent volumes accordingly.

2. Lysis and Binding
   2.1. Add **400 µl** of **RNA Blood Lysis and Binding Buffer** to each sample and mix (by pipette and/or vortex) and then let mixture sit at **ambient temperature** for **5 minutes**.
   2.2. Prior to each use, ensure **DNA/RNA Binding Beads** are evenly resuspended by mixing, and confirm no settled material remains at bottom of the bottle.
   2.3. Add **325 µl** of **DNA/RNA Binding Beads** to each sample and mix (by pipette and/or vortex) and then let sit at **ambient temperature** for **5 minutes**.
   2.4. Centrifuge samples for **5 minutes** at **4000 RPM**.
   2.5. Aspirate **875 µl** of **supernatant** and **discard** from each sample, leaving **50 µl** of sample remaining for continued processing.

3. Wash and Re-Bind
   3.1. Add **500 µl** of **Wash Buffer 1** to each sample and mix thoroughly to resuspend pellet.
   3.2. Allow beads to bind to magnet for **2 minutes** and then remove supernatant.

   3.3. Add **500 µl** of **80% Ethanol** to each sample and mix thoroughly to resuspend pellet.
   3.4. Allow beads to bind to magnet for **2 minutes** and then remove supernatant.

4. First Elution and DNASE Treatment
   4.1. Add **75 µl** of **Elution Buffer** to each sample mix sufficiently to ensure pellet is resuspended. Note that small aggregates may be observed.
   4.2. Hold samples at **ambient temperature** for **5 minutes** and then mix (by pipetting and/or vortex).
   4.3. Place samples on magnet and allow beads to settle toward magnet. Perform first elution by removing sample from binding beads and placing into new tube or 96-well plate position.
      4.3.1. If residual beads are present in eluted samples, use magnet to hold beads in place during transfer of samples.
   4.4. Add **75 µl** of **DNASE Solution** to each sample and mix by pipetting up and down **three times**, and then let sample sit at **ambient temperature** for **15 minutes**.

5. Second Wash and Final Elution
   5.1. Add **300 µl** of **RNA Binding and Lysis Buffer** and **243 µl** of **DNA/RNA Binding Beads** to each sample and mix (by pipetting and/or vortex) and then let mixture sit at **ambient temperature** for **5 minutes**.
   5.2. Add **500 µl** of **80% Ethanol** and mix thoroughly to resuspend pellet.
   5.3. Allow beads to bind to magnet for **2 minutes** and then remove supernatant.
   5.4. Add **500 µl** of **80% Ethanol** and mix thoroughly to resuspend pellet.
   5.5. Allow beads to bind to magnet for **2 minutes** and then remove supernatant.
   5.6. Add **150 µl** of **Elution Buffer** to each sample and mix sufficiently to ensure pellet is resuspended and incubate samples at **room temperature** for **5 minutes**.
   5.7. Hold samples at **ambient temperature** for **5 minutes** and then mix (by pipetting and/or vortex).
   5.8. Place samples on magnet and allow beads to settle toward magnet. Perform final elution by removing sample from binding beads and placing into destination tube or 96-well plate position.
      5.8.1. If residual beads are present in eluted samples, use magnet to hold beads in place during transfer of samples.
   5.9. Isolated RNA samples should be stored at -20°C for long-term storage.