Modified Blood Extraction Protocol

PRINCIPLE

This protocol describes a method for isolation of RNA/DNA from whole blood (0.1-0.5 mL) collected in PrimeStore MTM® (PS-MTM) using PrimeXtract spin columns.

STORAGE

The reagents and materials are stable for 18 months at ambient temperature (15-28 ºC) and 2 years stored refrigerated (4-10 ºC).

SPECIMEN

Whole blood (0.1 -0.5 mL) collected 3.0 mL tubes containing 1.5 mL of PS-MTM®. Alternatively, 0.1-0.3 mL of whole blood can be added to 2.0 mL tubes containing 1.0 mL of PrimeStore MTM®.

Product Use Limitations: PrimeXtract is intended extraction of high quality DNA/RNA from microbial samples with no claims for any specific clinical use. It is the user's responsibility to validate the performance of the PrimeXtract system for a specific use as determined by the established protocol for the user's laboratory. PrimeXtract is not intended for use in identifying a specific organism, or for clinical diagnostic purposes.

Blood Extraction and Purification Protocol:

Starting Material: Up to 0.5 mL of primary sample (whole blood) inactivated and stabilized in a standard 3.0 mL PrimeStore MTM collection tube (containing 1.5 mL of PrimeStore MTM).

1. Extraction is performed using the PrimeXtract Kit from Longhorn Vaccines & Diagnostics (San Antonio, TX). The liquid contents of the kit should be stored at +4ºC. Remove Micro Xtraction columns from the kit and store at ambient temperature (25ºC).
2. Before beginning extractions, warm Elution Solution (nuclease- free water) in a heating block set to 60-75ºC.
3. Vortex PrimeStore tube containing 0.1-0.5 mL whole blood for 3-5 minutes to suspend the sample.
4. Combine 200 µl of 100% ethanol (not provided), 200 µl of Lysis Buffer and 200 µl of sample (suspended PS-MTM + blood) into a clean 1.5 mL microcentrifuge tube (not provided). Vortex for 5 minutes on medium-high setting.
5. Microcentrifuge and spin at max setting (13.2 x 1000 rpm) for 1 minute to pellet debris.
6. Pipette liquid (minus pellet - ~600 uL) from step 5 into the extraction column/collection tube. Cap the column and place into microcentrifuge. Centrifuge for 60 seconds at 13.2 x 1000 rpm.
7. Remove extraction column from collection tube and discard flow through (eluate) into waste container. Place column back into collection tube. The nucleic acid is bound to the silica filter.
8. Add 500 µl of Wash Buffer 1 to the extraction column. Centrifuge for 1 minute at 13.2 x 1000 rpm. Remove tubes from centrifuge and discard flow through (eluate) into waste container. Place column back into collection tube.
9. Add 500 µl of Wash Buffer 2 to the extraction column. Centrifuge for 1 minute at 13.2 x 1000 rpm. Discard the flow through from collection tube. Place column back into collection tube.
10. Repeat procedure in step 9, to wash the filter twice with Wash Buffer 2.
11. Centrifuge extraction column for an additional 1 minute at 13.2 x 1000 rpm to remove trace amounts of Wash Buffers from filter.
12. Discard collection tube and transfer extraction column (with bound nucleic acid) into a sterile, 1.5 mL microcentrifuge tube (not provided).
13. Pipette 50 µl of Elution Solution (pre-heated to 60-75ºC) directly onto the silica filter in each column without touching the filter and incubate for 1 minute.
14. Centrifuge the columns for 1 minute at 13.2 x 1000 rpm. Remove and discard the column from the microcentrifuge tube.
15. Store nucleic acid at -20 to +4ºC until ready for use. For long-term storage, nucleic acid should be held at -80ºC.

PrimeXtract™ Reagent Safety and Health Precautions

PrimeXtract™ contains a proprietary blend of reagents that includes a chaotrophic compound (HMIS: Health Rating: 2, Physical Hazard: 0). These chemicals can be harmful if inhaled and/or ingested. PrimeXtract™ may cause irritation to skin, respiratory tract, and eyes. As with all chemicals, personal protective equipment (lab coat, gloves, safety goggles, etc.) should be used in accordance with the user's established laboratory safety guidelines.

First Aid Measures	
Skin Contacts	Get medical aid. Immediately flush skin with soap and water while removing contaminated clothing and shoes.
Eye Contact	Immediately flush eyes, occasionally lifting the upper and lower eyelids. Get medical aid immediately.
Inhalation	Remove from exposure to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.
Ingestion	Do not induce vomiting. If victim is conscious and alert, give 2-3 cups of water. Never give anything by mouth to an unconscious person. Get medical aid immediately.