TaqGen®

Product Manual

Taqgen® Pathogen DNA Enrichment & Purification

Cat no: PEM050

The Taqgen® Pathogen DNA Enrichment & Purification is suitable for the removal of Human DNA and purification of microbial DNA from liquid specimens, including whole blood, aspirates, cell culture, Synovial fluid, Pleural fluid, Cerebrospinal fluid, Ascites fluid, Pus, Nasal-wash fluid, Urine, Blood cultures.

For research use only.

Not for use in diagnostic procedures.

Description

The Taqgen® Pathogen DNA Enrichment & Purification is suitable for the removal of Human DNA and purification of microbial DNA from liquid specimens, including whole blood, aspirates, cell culture, Synovial fluid, Pleural fluid, Cerebrospinal fluid, Ascites fluid, Pus, Nasal-wash fluid, Urine, Blood cultures.

Product Components and Storage Conditions

Product	Storage condition	Total Vol	Vol/Reaction For 200 µL sample
RBC Lysis Buffer	RT	50 ml	600 µL
HgDNA Lysis Buffer	RT	30 ml	400 µL
Lysis Buffer	RT	30 ml	400 µL
Wash Buffer WB1	RT	10 ml conc	500 µL
Wash Buffer WB2	RT	10 ml conc	500 µL
Elution Buffer	RT	20 ml	50 µL
Spin Columns	RT	50 pc	1
Collection Tube	RT	50 pc	1

Reconstitution of buffers/reagents:

Buffer/Enzyme name	Procedure
Wash Buffer WB1 (10 ml conc)	Add 30 ml 100% Ethanol
Wash Buffer WB2 (10 ml conc)	Add 30 ml 100% Ethanol

Storage Conditions: Store the Taqgen® Pathogen DNA Enrichment & Purification Kit at 15°–30°C.

Safety Information: The Taqgen® Pathogen DNA Enrichment & Purification Kit contains guanidine hydrochloride, should be considered harmful and irritants.

Caution: Handle buffer with care. Bleach reacts with guanidine hydrochoride and should not be added to any sample waste.

Before You Begin

To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples and perform extractions from fresh material or material that has been immediately frozen and stored at -20 °C or -70 °C.

Protocol for Pathogen DNA Enrichment & Purification from Whole Blood (Clinical Sample).

1. **RBC Lysis**
   a. Add **600µL of RBC Lysis Buffer** to **200µL of whole blood**, mix by vortexing. mix thoroughly by vortexing or pipetting to obtain a uniform suspension. Incubate the sample for 5 min at room temperature. Centrifuge for 1 min at 14,000 rpm (20,000 x g). Decant supernatant.

   Note. If RBCs persist, resuspend the pellet in 600µL of RBC Lysis Buffer, mix thoroughly by vortexing and Incubate for 5 min at room temperature. Centrifuge for 1 min at 14,000 rpm (20,000 x g). Decant supernatant. Using larger sample volume >500µL of blood may require twice RBC lysis step.
2. **Human cell Lysis**
   a. Add **400µL HgDNA Lysis Buffer** to pellet mix thoroughly by vortexing or pipetting to obtain a uniform suspension. Incubate the sample for 5 min at room temperature. Centrifuge for 1 min at 14,000 rpm (20,000 x g). Decant supernatant.
3. **Microbial cell Lysis**
   a. Add **400µL Lysis Buffer** to pellet, mix thoroughly by vortexing or pipetting to obtain a uniform suspension. Incubate the sample for 56°C for 10 min.
   b. Add 200µL of 100% ethanol. Mix thoroughly by vortexing.
4. **Bind DNA**
   c. For each sample, place one **SpinColumn** into a Collection Tube. Apply the sample to the column. Centrifuge for **1 min at 8000 rpm (6000 x g)**.Discard the filtrate and reuse the collection tube.

   Note: Gently transfer the lysate/binding buffer mix (~600 µl) to a gDNA Purification Column pre-inserted into a collection tube, without touching the upper column area. Avoid touching the upper have formed during lysis. Any material that touches the upper area of the column, including any foam, may lead to salt contamination in the eluate
5. **Wash and dry silica membrane**
   a. Add **500µL Wash Buffer WB1**. Close the cap and invert a few times, so that the wash buffer reaches the cap. Centrifuge for **1 min at 8000 rpm (6000 x g)**. Discard the filtrate.
   b. Add **500µL Wash Buffer WB2**. Centrifuge **1 min for 8000 rpm (6000 x g)**. Discard the filtrate.
   c. Centrifuge for **2 min at 14,000 rpm (20,000 x g).**
   Note: Residual ethanol is removed during this step.
6. **Elute DNA**
   a. Place the Spin Column into a 1.5 mL microcentrifuge. Add **50µL Elution Buffer**. Incubate at room temperature for 1 min. Centrifuge **1 min at 8000 rpm (6000 x g).**