Product Manual

Taqgen® Blood Genomic DNA Kit
20 preps
Cat no: MSB120

The **Taqgen® Blood Genomic DNA Kit** is suitable for Genomic DNA Extraction from whole Blood, body fluids, buccal swabs, buffy coat and avian blood.

For research use only.

Not for use in diagnostic procedures.

Description

The Taqgen® Blood Genomic DNA kit, a spin column based kit, provide an easy, rapid and efficient purification of high quality genomic DNA from whole blood and body fluid. The purified DNA can be used directly in a variety of downstream applications, including PCR, RealTime PCR, southern blotting and restriction enzyme digestion. The kit eliminates the need for expensive resin, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation. The standard procedure takes less than 20 minutes following cell lysis and yield purified DNA greater than 30 Kb in size. This kit allows for the single or multiple simultaneous processing of samples.

Product Components and Storage Conditions

Product	Storage condition	Total Vol	Vol/Reaction For 200 µL sample
Proteinase K	2°–8°C	400µL	20 µL
Lysis Buffer	RT	20 ml	400 µL
Wash Buffer WB1	RT	8 ml conc	500 µL
Wash Buffer WB2	RT	4 ml conc	500 µL
Elution Buffer	RT	20 ml	100 µL
Spin Columns	RT	20 pc	1
Collection Tube	RT	20 pc	1

Reconstitution of buffers/reagents:

Buffer/Enzyme name	Procedure
Proteinase K Lyophilized (5mg)	Add 200 µL Proteinase buffer
Wash Buffer WB1 (8 ml conc)	Add 6 ml 100% Ethanol
Wash Buffer WB2 (4 ml conc)	Add 10 ml 100% Ethanol

Storage Conditions: Store the Taqgen® Blood Genomic DNA Kit at 15°–30°C except the proteinase K after reconstitute.

Safety Information: The Taqgen® Blood Genomic DNA kit contains guanidine hydrochloride and proteinase K, 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. Buffers For sample volume adjustment: PBS:137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4, pH 7.4.

TE buffer: 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

Protocol for Whole Blood Genomic DNA Purification

1. **Lyse Cells**
   a. Add **20µL of Proteinase K Solution** to **200µL of whole blood**, mix by vortexing. Add **400µL of Lysis Buffer**, mix thoroughly by vortexing or pipetting to obtain a uniform suspension
   Note. If using less than 200µL of blood, adjust sample volume to 200µL with 1X PBS or TE buffer (not provided).
   b. Incubate at 56°C for 10 min in a water bath. while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed.
2. **Adjust DNA binding conditions**
   a. Add **200µL of ethanol** (96-100%).
   Note: Adjust the volume of ethanol required based on the amount of starting material. Vortex to mix thoroughly
3. **Bind DNA**
   a. For each sample, place one **Spin Column** into a Collection Tube. Apply the sample to the column. Centrifuge for **1 min** at **11,000 x *g***. Discard the filtrate and reuse the collection tube.
4. **Wash and dry silica membrane**
   a. Add **500µL Wash Buffer WB1** (with Ethanol Added).Centrifuge for **1 min** at **11,000 x *g***. Discard the filtrate.
   b. Add **500µL Wash Buffer WB2** (with ethanol added).Centrifuge for **1 min** at **11,000 x *g***. Discard the filtrate.
   c. Centrifuge for **2 min** at **11,000 x *g***.
   Note: Residual ethanol is removed during this step.
5. **Elute DNA**
   a. Place the Spin Column into a 1.5 mL microcentrifuge. Add **100µL Elution Buffer.** Incubate at room temperature for 1 min. Centrifuge **1 min** at **11,000 x *g***.

Protocol for DNA Purification from Large Volumes of Whole Blood

1. **Sample Preparation**
   a. Add 1mL of ice cold nuclease free water to 500µL of whole blood, mix thoroughly by vortexing or pipetting.
   b. Incubate the sample for 5 min at room temperature.
   c. Centrifuge for 5 min at 3,000 rpm.
   d. Discard the supernatant.
   e. Resuspend the pellet in 200µL of 1 x PBS.
   f. Proceed to step 1 of the Whole Blood Genomic DNA Purification Protocol.
   Note. For purification of DNA from samples exceeding the standard 200µL volume, it is necessary to burst red blood cells prior to performing the cell lysis step. Up to 500 µL of mammalian blood can be processed using following protocol

Protocol for DNA Purification from Nucleated Blood

1. Sample Preparation

   a. Take 2-10 µL of nucleated blood**.**
   b. Adjust the volume to 200µL with 1×PBS
   c. Proceed to step 1 of the Whole Blood Genomic DNA Purification Protocol
   Note: Nucleated avian or fish blood contains very large amounts of genomic DNA and therefore the volume of the staring material has to be scaled down. The DNA purification procedure follows the same protocol as mammalian blood, except that 2-10 µL of blood are used per purification.

Protocol DNA Purification from Buccal Swabs

1. **Sample Preparation**
   a. To collect a sample, scrape the swab 5-6 times against the inside cheek
   b. Swirl the swab for 30-60 s in 200 µL of 1xPBS.
   c. Go to step 1 of the standard Whole Blood Genomic DNA Purification Protocol

Protocol for DNA Purification from Bone Marrow

1. **Sample Preparation**
   a. Harvest 25-200 µL of fresh or frozen bone marrow.
   b. Adjust the volume to 200 µL with 1×PBS
   c. Proceed to step 1 of the Whole Blood Genomic DNA Purification Protocol

Protocol for DNA Purification from Buffy Coat

1. **Sample Preparation**
   a. Centrifuge 1.5 mL of whole blood at 5,000 rpm for 10 min at room temperature. Three layers should be visible.
   b. Remove upper clear layer by aspiration
   c. Collect approximately 200 µL of intermediate layer using an automatic pipette.
   Note. If necessary, adjust the volume to 200 µL with 1×PBS
   d. Proceed to step 1 of the Whole Blood Genomic DNA Purification Protocol.

Protocol for DNA Purification from Bone Marrow

1. **Sample Preparation**
   a. Add 0.5 mL of 0.5 M EDTA to 4.5 mL of urine (final concentration 50 mM).
   b. Centrifuge 10 min at 3000 rpm.
   c. Discard the supernatant.
   d. Resuspend the pellet in 200 µL 1×PBS.
   e. Proceed to step 1 of the Whole Blood Genomic DNA Purification Protocol

Protocol for DNA Purification from Dried Blood Spots

1. **Sample Preparation**
   a. Cut out the section of filter containing the dried blood sample and place into a microcentrifuge tube.
   b. Add 200 µL of 1×PBS and Incubate 5-10 min at room temperature.
   c. Proceed to step 1 of the Whole Blood Genomic DNA Purification Protocol
   Note: Taqgen® Tissue DNA kit ensures the efficient purification for the Dried Blood cards.