TaqGen®

# Product Manual

## Taqgen® Whole Blood Genomic DNA Purification Kit 50 preps
## Cat no: MTB050

The **Taqgen® Whole Blood Genomic DNA Purification Kit** is suitable for Genomic DNA Extraction from whole Blood, body fluids, buccal swabs, buffy coat and Cultured cells.

For research use only.

Not for use in diagnostic procedures.

**Description**

The Taqgen® Whole Blood Genomic DNA Purification 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 | 1.2 mL | 20 µL |
| Lysis Buffer | RT | 25 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 | 100 µL |
| Spin Columns | RT | 50 pc | 1 |
| Collection Tube | RT | 50 pc | 1 |

**Reconstitution of buffers/reagents:**

| Buffer/Enzyme name | Procedure |
|---|---|
| Proteinase K Lyophilized (20mg) | Add 1.2 ml Proteinase buffer |
| 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® Whole Blood Genomic DNA Purification kit at 15°–30°C except the proteinase K after reconstitute (2°–8°C).

**Safety Information:** The Taqgen® Whole Blood Genomic DNA Purification 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
   b. Incubate at **room temperature for 10 min**.
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 for each sample, place one Spin Column 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 (~800 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.
4. **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.
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 8000 rpm (6000 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.