Geneaid™ DNA Isolation Kit (Blood) Quick Protocol

For research use only

Catalogue Number

GEB003, GEB100, GEB01K, GEB01K+,

Instruction Manual Download

When using this product for the first time, or if you are unfamiliar with the procedure, please scan the QR code and download the complete instruction manual.

Instruction Manual Download

300 µl Whole Blood Protocol Procedure

1. RBC Lysis

Transfer **900 µl of RBC Lysis Buffer and 300 µl of whole blood** into a 1.5 ml microcentrifuge tube then mix by inverting. Do not vortex. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte pellet. Carefully remove the supernatant, retaining approximately 50 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.

2. Lysis

Add **300 µl of Cell Lysis Buffer** to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60ºC incubation, add 1.5 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 5 minutes.

3. Protein Removal

Add **100 µl of Protein Removal Buffer** to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 14-16,000 x g for 3 minutes to form a tight, dark brown, protein pellet. If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 14-16,000 x g for another 3 minutes.

4. DNA Precipitation

Transfer the supernatant to a clean 1.5 ml microcentrifuge tube then add **300 µl of isopropanol** and mix well by gently inverting 20 times. Centrifuge at 14-16,000 x g for 5 minutes then carefully discard the supernatant and add **300 µl of 70% ethanol** to wash the pellet. Centrifuge at 14-16,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add **100 µl of DNA Hydration Buffer** then gently vortex for 10 seconds. Incubate at 60ºC for 5-10 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

3 ml Whole Blood Protocol Procedure

1. RBC Lysis

Transfer **9 ml of RBC Lysis Buffer and 3 ml of whole blood** into a 15 ml centrifuge tube then mix by inverting. Do not vortex. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte pellet. Carefully remove the supernatant, retaining approximately 300 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.

2. Lysis

Add **3 ml of Cell Lysis Buffer** to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60ºC incubation, add 15 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 5 minutes.

3. Protein Removal

Add **1 ml of Protein Removal Buffer** to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 2-3,000 x g for 5 minutes to form a tight, dark brown, protein pellet. If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 3-6,000 x g for another 5 minutes.

4. DNA Precipitation

Transfer the supernatant to a clean 15 ml centrifuge tube then add **3 ml of isopropanol** and mix well by gently inverting 20 times. Centrifuge at 2-3,000 x g for 5 minutes then carefully discard the supernatant and add **3 ml of 70% ethanol** to wash the pellet. Centrifuge at 2-3,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add **300 µl of DNA Hydration Buffer** then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

10 ml Whole Blood Protocol Procedure

1. RBC Lysis

Transfer **30 ml of RBC Lysis Buffer and 10 ml of whole blood** into a 50 ml centrifuge tube then mix by inverting. Do not vortex. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte pellet. Carefully remove the supernatant, retaining approximately 300 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.

2. Lysis

Add **10 ml of Cell Lysis Buffer** to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60ºC incubation, add 50 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 10 minutes.

3. Protein Removal

Add **3.33 ml of Protein Removal Buffer** (add 4.5 ml for compromised blood samples) to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 2-3,000 x g for 5 minutes to form a tight, dark brown, protein pellet. If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 3-6,000 x g for another 5 minutes.

4. DNA Precipitation

Transfer the supernatant to a clean 50 ml centrifuge tube then add **10 ml of isopropanol** (add 13.5 ml for compromised blood samples) and mix well by gently inverting 20 times. Centrifuge at 2-3,000 x g for 5 minutes then carefully discard the supernatant and add **10 ml of 70% ethanol** to wash the pellet. Centrifuge at 2-3,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add **1 ml of DNA Hydration Buffer** (add 500 µl for compromised blood samples) then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

Components

Geneaid™ Blood Kit	GEB003	GEB100	GEB01K	GEB01K+
Volume of blood processed per kit	3 ml	100 ml	1000 ml	1000 ml
RBC Lysis Buffer	12 ml	360 ml	500 ml x 7	500 ml x 7
Cell Lysis Buffer	3 ml	100 ml	500 ml x 2	500 ml x 2
Protein Removal Buffer	1 ml	40 ml	400 ml	400 ml
DNA Hydration Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	1 ml	50 ml	500 ml	500 ml
RNase A (10 mg/ml)	25 µl	550 µl	Not included	5 ml

Storage

RBC Lysis Buffer, Cell Lysis Buffer, Protein Removal Buffer, DNA Hydration Buffer should be stored dry at room temperature (15-25ºC). RNase A should be stored at 4ºC for extended periods.