Geneaid™ DNA Isolation Kit (Cell) Quick Protocol

For research use only

Catalogue Number

GEC005, GEC150, GEB1.5K, GEB1.5K+,

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

3-5 x 10^6 Cultured Cell Protocol Procedure

1. Sample Preparation

Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)

Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells detach add the medium then transfer to a 1.5 ml microcentrifuge tube. Proceed with Suspension Cultured Animal cells.

Suspension Cultured Animal Cells

Transfer cells (3-5 x 10^6) to a 1.5 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant retaining approximately 50 µl of residual buffer and cell pellet. Vortex the tube until the cell pellet is completely resuspended in the residual buffer.

2. Lysis

Add **600 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 3 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 **200 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 pellet.

NOTE: 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 **600 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 **600 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 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

3-5 x 10^7 Cultured Cell Protocol Procedure

1. Sample Preparation

Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)

Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells detach add the medium then transfer to a 15 ml centrifuge tube. Proceed with Suspension Cultured Animal cells.

Suspension Cultured Animal Cells

Transfer cells (3-5 x 10^7) to a 15 ml centrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant retaining approximately 50 µl of residual buffer and cell pellet. Vortex the tube until the cell pellet is completely resuspended in the residual buffer.

2. Lysis

Add **6 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 30 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 **2 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 pellet.

NOTE: 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 **6 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 **6 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 **200 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.

Components

Geneaid™ Cultured Cell Kit	GEC005	GEC150	GEC01.5K	GEC01.5K+
Number of cells processed per kit	**2 x 10^7**	**6 x 10^8**	**6 x 10^9**	**6 x 10^9**
Cell Lysis Buffer	3 ml	100 ml	1000 ml	1000 ml
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

Cell Lysis Buffer, Protein Removal Buffer, DNA Hydration Buffer should be stored dry at room temperature (15-25ºC) for up to 2 years. RNase A should be stored at 4ºC for extended periods.