KIAGENE FANAVAR

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Cell And Tissue DNA Isolation Kit

Cat. No:

FPKT023.0025
FPKT023.0050
FPKT023.0100

Contents:

Components	25 preps	50 preps	100 preps
DT1 Buffer	6 ml	12 ml	24 ml
DT2 Buffer	6 ml	12 ml	24 ml
DT3 Buffer	7.5 ml	15 ml	30 ml
Wash Buffer	6 ml	12 ml	12 ml ×2
Elution Buffer	2 ml	4 ml	8 ml
Proteinase K 20mg/ml	250 µl	500 µl	1000 µl
RNase A 10mg/ml	100 µl	200 µl	400 µl
Column and collection tubes	25 each	50 each	50 each×2

Kit storage:

⚠ This kit should be stored at room temperature.

⚠ **Proteinase K** should be stored at -20 °C.

⚠ If properly stored, all kit components are stable until the expiration date printed on the label.

⚠ After adding **Ranse** to the **DT1 buffer** and **absolute ethanol** to **wash buffers**, Store the **DT1** and **wash Buffers** at +2 to +8°C

Additional Equipment and Reagent required

•Absolute ethanol

• Standard tabletop microcentrifuge capable of 13,000 x g centrifugal force

• Microcentrifuge tubes, 1.5 ml, sterile

Application

This kit provides a convenient and rapid method to isolate total DNA from cell culture And Tissue samples. The purified DNA is of the highest quality and is fully compatible with all downstream applications such as PCR, qPCR, NGS, and microarrays since all humic acid substances and other PCR inhibitors are removed during the isolation process.

The procedure is optimized to achieve reliable results within **60 min**.

Handling Requirements and Safety Information

⚠ All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.

⚠ DT3 Buffer contains guanidinium hydrochloride which is an irritant.

⚠ Do not allow DT2 Buffer and DT3 Buffer to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water.

⚠ If you spill the reagent, dilute the spill with water before wiping it up.

⚠ Do not use any modified ethanol.

⚠ Do not pool reagents from different lot numbers.

⚠ Immediately after usage, close all bottles to avoid leakage, varying buffer concentrations, or buffer conditions.

⚠ After first opening store all bottles in an upright position.

⚠ Do not allow the DT2 Buffer and DT3 Buffer to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce highly toxic gas.

⚠ Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.

⚠ Do not contaminate the reagents with bacteria, viruses, or nucleases. Use disposable pipets and nuclease-free pipet tips to remove aliquots from reagent bottles.

preparation procedure:

Working Solution Preparation

⚠ **add RNase A to DT1 Buffer completely Dissolve the RNase A by vortexing. store the DT1 buffer at 4 °C.**

FPKT023.0025-25 preps

⚠ **Add 5 ml absolute ethanol** to DT3 Buffer Bottle.

⚠ **Add 24 ml of absolute ethanol** to the Wash Buffer Bottle.

FPKT023.0050-50 preps

⚠ **Add 10 ml absolute ethanol** to DT3 Buffer Bottle.

⚠ **Add 48 ml of absolute ethanol** to the Wash Buffer Bottle.

FPKT023.0100-100 preps

⚠ **Add 20 ml absolute ethanol** to DT3 Buffer Bottle.

⚠ **Add 48 ml of absolute ethanol** to each Wash Buffer Bottle.

Please note that the Ethanol concentration of a Washing Buffer may decrease during long-term storage resulting in a drop-down of the final DNA yield.

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⚠ before starting Incubate the **Elution buffer at 55 to 65 ° C** until the end of the protocol to obtain the maximum yields.

Protocol

1. Sample preparation

1a. Preparation for Isolation of DNA from Cultured Cells (suited for 1~5× 10^6 cells): Pellet the cells from cell culture by centrifuge in **3000×g for 5 min**. Discard the supernatant (about **10^6 cells**). Proceed with step 2.

1b. Preparation for Isolation of DNA from Tissues: Thoroughly homogenize the tissue mechanically using a homogenizer Alternatively, grind the tissue to a fine powder with liquid nitrogen in a precooled mortar or cut the tissue into small pieces on a sterile petri dish by a scalpel. For frozen tissue samples stored in 20% glycerol or 20% DMSO, pellet the sample by centrifugation, and discard the supernatant before homogenization. Cutting the tissue into small pieces increases the yield of genomic DNA and reduces lysis incubation time. **Transfer 20 mg of homogenized tissue to a 2 ml tube**. Do not use more than 20 mg of tissue. Proceed with step 2.

2. Add **200 µl DT1 Buffer**. Resuspend by **vortex for 15 s**. **Incubate at 37ºC for 30 minutes**. During incubation, invert the tube occasionally.

3. Add **10 µl of Proteinase K** then mix by vortex. **Incubate at 60ºC for at least 15 minutes**. During incubation, invert the tube every 3 minutes.

4. Add **200 µl DT2 Buffer** to the microtube and mix them by invert.

5. Add **200 µl of absolute ethanol** to the sample and mix by **vortex for 10 seconds**.

6. Centrifuge in **8000-10000×g for 1 min** (to pellet the remaining cells).

7. Transfer the supernatant into a DNA spin column assembled in a clean collection tube (provided). Centrifuge at **10000×g for 1 min** at room temperature.

8. Disconnect the DNA spin column from the collection tube and discard the flow-through solution. Reconnect the DNA spin column to the collection tube.

9. Add **500 µl DT3** to the column and **centrifuge (10000×g, 1 min).** Discard flow through.

10. Add **700 µl wash buffer** to the column and **centrifuge (10000×g, 1 min).** Discard flow through.

11. Centrifuge the DNA spin column at **10000×g for 1 min** to remove residual ethanol.

12. Place the DNA spin column into the clean new microtube. Add 30 µl of pre-warmed Elution Buffer or sterile DDW directly onto the column membrane and stand for 3 min. Centrifuge at **10000×g for 1 min**.

13. Add another **30 µl pre-warmed Elution Buffer** or sterile DDW directly onto the column membrane and stand for 3 min. Centrifuge at **10000×g for 1 min**.

14. Check **7 µl of extracted** DNA by 0.5 % agarose gel electrophoresis. **Store DNA at 4˚C or -20˚C**.