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DualPrep DNA/RNA Isolation Kit (DPDRI)
Catalog #MB6908-50
50 Preps
or
Catalog #MB6908-100
100 Preps

Description

ScienCell's DualPrep DNA/RNA Isolation Kit provides a fast and reliable way to purify both genomic DNA and total RNA from a single biological sample (e.g., human or animal tissue or cultured cell samples). The sample lysate is passed through DNA and RNA spin columns in sequence for the selective isolation of genomic DNA and total RNA, respectively. The purified DNA is suitable for downstream applications such as PCR, Southern blot analysis, and genotyping. The purified total RNA is suitable for downstream applications such as cDNA synthesis, microarray, RT-qPCR, RNA-Seq, and Northern blot analysis.

Kit Components

Cat #	Item	Quantity
MB6908a-1	Buffer CL	35 mL
MB6908b-1	Buffer DW1	12 mL
MB6908c-1	Buffer DW2	9 mL
MB6908d-1	Buffer RW3	34 mL
MB6908e-1	Buffer RW4	13 mL
MB6908f-1	Buffer DE	5 mL
MB6908g-1	Nuclease-free water	4 mL
MB6908h	DNA spin columns	50 pieces
MB6908i	RNA spin columns (in wash tubes)	50 pieces
MB6908j	Wash tubes	150 pieces

or

Cat #	Item	Quantity
MB6908a-2	Buffer CL	65 mL
MB6908b-2	Buffer DW1	24 mL
MB6908c-2	Buffer DW2	17 mL
MB6908d-2	Buffer RW3	63 mL
MB6908e-2	Buffer RW4	26 mL
MB6908f-2	Buffer DE	10 mL

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MB6908g-2	Nuclease-free water	8 mL
MB6908h	DNA spin columns	100 pieces
MB6908i	RNA spin columns (in wash tubes)	100 pieces
MB6908j	Wash tubes	300 pieces

Materials Required (Not Provided)

β-mercaptoethanol
Dithiothreitol, 2 M in MiliQ water
Ethanol (96-100%)
70% Ethanol in water
RNA stabilization reagent, such as StableRNA Storage Solution (ScienCell, Cat #MB6408)
1.7 mL (or 1.5 mL) microcentrifuge tubes (DNase/RNase free)

Quality Control

The yield of purified genomic DNA and total RNA from 5 x 10^6 cultured human primary cells using DPDRI was analyzed by spectrophotometry. The free of genomic DNA contamination in purified total RNA fraction was verified by qPCR.

Product Use

DPDRI is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

Ambient temperature.

Reagent Preparation

1. Buffer DW1 (12 mL, Cat #MB6908b-1 or 24 mL, Cat #MB6908b-2) is provided as a concentrate. Prior to use for the first time, add **16 mL** ethanol (96-100%) to 12 mL, Cat #MB6908b-1 to make complete buffer DW1, or add **32 mL** ethanol (96-100%) to 24 mL, Cat #MB6908b-2 to make complete buffer DW1. Mix well. Keep container tightly closed and store at room temperature.

2. Buffer DW2 (9 mL, Cat #MB6908c-1 or 17 mL, Cat #MB6908c-2) is provided as a concentrate. Prior to use for the first time, add **22 mL** ethanol (96-100%) to 9 mL, Cat #MB6908c-1 to make complete buffer DW2, or add **40 mL** ethanol (96-100%) to 17 mL, Cat #MB6908c-2 to make complete buffer DW2.Mix well. Keep container tightly closed and store at room temperature.

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3. Buffer RW3 (34 mL, Cat #MB6908d-1 or 63 mL, Cat #MB6908d-2) is provided as a concentrate. Prior to use for the first time, add **8 mL** ethanol (96-100%) to make complete buffer RW3, or add **15 mL** ethanol (96-100%) to make complete buffer RW3. Mix well. Keep container tightly closed and store at room temperature.

4. Buffer RW4 (13 mL, Cat #MB6908e-1 or 26 mL, Cat #MB6908e-2) is provided as a concentrate. Prior to use for the first time, add **44 mL** ethanol (96-100%) to make complete buffer RW4, or add **88 mL** ethanol (96-100%) to make complete buffer RW4. Mix well. Keep container tightly closed and store at room temperature.

Procedures

Note: Avoid touching the DNA/RNA spin column membranes with the tip-end of pipettes.

1. Prepare cultured cell samples as stated in step 1a or tissue samples as stated in step 1b:

   1a. Collect up to 5 x 10^6 cells from cultures either in suspension or in monolayer. proceed to Step 2 or freeze at -80°C until use.
   Note: Harvesting of more than 5 x 10^6 cells may lead to genomic DNA contamination in the purified total RNA fraction.
   Note: culture medium should be removed completely to ensure high nucleic acid recovery rate and purity.

   1b. Excise up to 30 mg of tissue sample and proceed immediately to Step 2. ***Optional:*** RNA Stabilization Reagent (e.g., StableRNA Storage Solution, ScienCell, Cat #MB6408) may be used to protect RNA from degradation during tissue handling.

2. Add 600 µL of Buffer CL (Cat #MB6908a) to sample. Disrupt and homogenize sample with a homogenizer or a syringe and 22-gauge needle. Centrifuge the lysate at ≥ 15000 x *g* for 3 minutes.
   Note: for RNase-rich samples, prior to use, add 6 µL of β-mercaptoethanol or 12 µL of 2 M dithiothreitol to 600 µL of Buffer CL.

3. Place DNA spin column (Cat #MB6908h) in a Wash tube (Cat #MB6908j). Transfer the supernatant from Step 2 to the DNA spin column in the wash tube. Centrifuge for 30 seconds at ≥8,000 x *g*.

4. For total RNA purification, proceed to Step 6 and store the DNA spin column in a new wash tube at room temperature or 4°C if DNA purification is anticipated. Long storage time for over 4 hours is not recommended.

5. If total RNA purification is not desired, proceed to Step 14 directly for genomic DNA purification.

Total RNA Purification

6. Add 600 µL 70% ethanol to the flow-through from Step 3. Mix thoroughly by pipetting.

7. Transfer the mixture (up to 650 µL each time) to the RNA spin column in a wash tube (Cat #MB6908i). Close the cap and centrifuge at ≥8,000 x *g* for 30 seconds. Discard the filtrate and put the spin column back to the same wash tube. Repeat this step until all of the mixture has been applied to the spin column.

8. Add 700 µL of complete Buffer RW3 (Cat #MB6908d-1 or Cat #MB6908d-2) to the RNA spin column. Close the cap and centrifuge at ≥8,000 x *g* for 30 seconds. Discard the filtrate and put the spin column back to the same wash tube.

9. Add 500 µL complete Buffer RW4 (Cat #MB6908e-1 or Cat #MB6908e-2) to the RNA spin column. Close the cap and centrifuge at ≥8,000 x *g* for 30 seconds. Discard the filtrate and put the spin column back to the same wash tube.

10. Repeat Step 9 once.

11. Place the RNA spin column in a new wash tube (Cat #MB6908j). Centrifuge at ≥ 15000 x *g* for 2 minutes. Discard the wash tube containing the filtrate and transfer the spin column into a new 1.7 mL microcentrifuge tube.

12. Add 50 µL Nuclease Free Water (Cat #MB6908g) directly to the spin column membrane. Centrifuge for 1 minute at ≥8,000 x *g* to elute RNA.

13. ***Optional:*** If a higher yield of total RNA is required, repeat step 12 with another 50 µL of nuclease-free water (Cat #MB6908g) and combine the two eluents. The final RNA concentration will be lower.

Genomic DNA Purification

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1. Add 500 µL of complete Buffer DW1 (Cat #MB6908b-1 or Cat #MB6908b-2) to the DNA spin column in a wash tube from Step 4. Close the cap and centrifuge at ≥8,000 x *g* for 30 seconds. Discard the filtrate and put the spin column back to the same wash tube.

2. Add 500 µL complete Buffer DW2 (Cat #MB6908c-1 or Cat #MB6908c-2) to the DNA spin column (reuse wash tube from previous step). Close the cap, and centrifuge at ≥8,000 x *g* for 30 seconds. Discard the filtrate and put the spin column back to the same wash tube.

3. Place the DNA spin column in a new wash tube (Cat #MB6908j). Centrifuge at ≥ 15000 x *g* for 2 minutes. Discard the wash tube containing the filtrate and transfer the spin column into a new 1.7 mL microcentrifuge tube.

4. Add 80 µL Buffer DE (Cat #MB6908f) directly to the spin column membrane. Incubate at room temperature for 1 minute. Centrifuge for 1 minute at ≥8,000 x *g* to elute DNA.

5. ***Optional:*** If higher yield of DNA is required, repeat step 17 with another 80 µL of Buffer DE (Cat #MB6908f) and combine the two eluents. The final DNA concentration will be lower.