Rev. 2

SpeeDNA Isolation Kit
(SPDNAI)
Catalog #MB6918-1
100 Preps
or
Catalog #MB6918-2
200 Preps

Description

ScienCell SpeeDNA Isolation Kit provides a fast and reliable way to purify total DNA (e.g., genomic, mitochondrial and pathogen) from biological samples including whole blood, plasma, serum, human or animal tissue or cultured cell. The SPDNAI isolates DNA with minimal handling while excluding organic denaturants. The buffer system is optimized Purified DNA is suitable for downstream applications such as PCR, Southern blot analysis, and genotyping.

Kit Components

Cat #	Item	Quantity
MB6918a-1	Buffer CLT	24 mL
MB6918b-1	Buffer DW1	24 mL
MB6918c	Buffer DW2	37 mL
MB6918d-1	Buffer DE	10 mL
MB6918e	DNA spin columns	100 pieces
MB6918f	Wash tubes	200 pieces
MB6918g	Protease K	1.2 mL x2

or

Cat #	Item	Quantity
MB6918a-2	Buffer CLT	48 mL
MB6918b-2	Buffer DW1	48 mL
MB6918c	Buffer DW2	37 mL x2
MB6918d-2	Buffer DE	20 mL
MB6918e	DNA spin columns	200 pieces
MB6918f	Wash tubes	400 pieces
MB6918g	Protease K	1.2 mL x4

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Materials Required (Not Provided)

1x PBS solution
Ethanol (96-100%)
1.7 mL (or 1.5 mL) microcentrifuge tubes (DNase/RNase free)

Quality Control

The yield of purified genomic DNA from 5 x 10^6 cultured human primary cells using SPDNAI was analyzed by spectrophotometry.

Product Use

SPDNAI 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 (24 mL, Cat #MB6918b-1, or 48 mL, Cat #MB6918b-2) is provided as a concentrate. Prior to use for the first time, add **32 mL** ethanol (96-100%) to the provided 24 mL of concentrate (Cat #MB6918b-1 for 100 preps) to make complete buffer DW1, or add **64 mL** ethanol (96-100%) to the provided 48 mL of concentrate (Cat #MB6918b-2 for 200 preps) to make complete buffer DW1. Mix well. Keep container tightly closed and store at room temperature.

2. Buffer DW2 (37 mL, Cat #MB6918c) is provided as a concentrate. Prior to use for the first time, add **90 mL** ethanol (96-100%) to the provided 37 mL of concentrate (Cat #MB6918c) to make complete buffer DW2. Mix well. Keep container tightly closed and store at room temperature.

Procedures

Note: Avoid touching the DNA spin column membranes with pipet tips.

1. Prepare biological sample as follows, based on sample type (e.g., for whole blood, serum, and plasma samples, follow Step 1a; for cultured cell samples, follow Step 1b; for tissue samples, follow Step 1c):

    1a. **Whole blood, serum, or plasma samples.** Aliquot up to 100 µL whole blood, serum, or plasma sample and process to Step 2a immediately or freeze at -80ºC until use.

1b. **Cultured cell samples.** Collect up to 5 x 10^6 cells from cultures either in suspension or in monolayer. Proceed to Step 2b or freeze at -80°C until use. **Note:** Culture medium should be removed completely to ensure high nucleic acid recovery rate and purity.

1c. **Tissue samples**. Excise up to 30 mg of tissue sample and proceed immediately to Step 2b.

2. Lyse cells as follows, based on sample state (e.g., for whole blood, serum, and plasma samples, follow Step 2a; for cultured cell samples, follow Step 2b; for tissue samples, follow Step 2c):

   2a. **Whole blood, serum, or plasma samples.** Add 20 µL of Protease K (Cat #MB6918g) to sample. Adjust volume to 220 µL with 1 x PBS solution. Add 200 µL buffer CLT (Cat #MB6918a). Mix by vertexing. Proceed to step 3.

   2b. **Cultured cell samples.** Resuspend cell samples using 1 x PBS solution. Add 20 µL of Protease K (Cat #MB6918g) to sample. Add 200 µL buffer CLT (Cat #MB6918a). Mix by vertexing. Proceed to step 3.

   2c. **Tissue samples.** Add 200 µL of Buffer CLT (Cat #MB6918a) to sample and incubate at 56 °C for 10 minutes. Add 20 µL of Protease K (Cat #MB6918g) and 200 µL 1 x PBS solution to sample. Disrupt and homogenize sample with a homogenizer or a syringe and 22-gauge needle. Proceed to step 3.

3. Incubate sample at 56 °C for 10 minutes.

4. Add 200 µL ethanol (96-100%). Mix by vertexing.

5. Place DNA spin column (Cat #MB6918e) in to a wash tube (Cat #MB6908f). Transfer sample to the DNA spin column in wash tube. Centrifuge for 1 minute at ≥8,000 x *g*. Discard flow through and return column to emptied wash tube.

6. Add 500 µL of complete Buffer DW1 to the DNA spin column from previous step. Close the cap and centrifuge at ≥8,000 x *g* for 1 minute. Discard the flow through and reuse emptied wash tube.

7. Add 600 µL complete Buffer DW2 to the DNA spin column. Centrifuge at ≥8,000 x *g* for 1 minute. Discard the flow through and reuse emptied wash tube.

8. ***Optional:*** Add another 600 µL complete Buffer DW2 to the DNA spin column. Centrifuge at ≥8,000 x *g* for 1 minute. Discard the flow through.

9. Place the DNA spin column in a new wash tube (Cat #MB6918f). Centrifuge at ≥15,000 x *g* for 2 minutes. Discard the wash tube containing the flow through and transfer the spin column to a new 1.7 mL microcentrifuge tube (not supplied).

10. Add 80 µL Buffer DE (Cat #MB6918d) 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.

    Optional: If higher yield of DNA is required, repeat Step 9 with another 80 µL of Buffer DE and combine the two eluants. The final DNA concentration will be lower.