CYDN16-CHANGYU Apoptotic DNA Ladder Kit

Catalog number	Package
CYDN1601	20 preps
CYDN1602	50 preps

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature. Lysis/Binding Buffer CB may precipitate at low temperature; redissolve in a 37 °C water bath for a few minutes until clear and cool to room temperature before use. Keep all solutions tightly capped after use to avoid evaporation, oxidation and pH drift.

Applications

For rapid extraction of apoptotic DNA ladder.

Kit components

Component	Storage	20 preps (CYDN1601)	50 preps (CYDN1602)
Lysis/Binding Buffer CB	15-25 °C	6 ml	15 ml
Wash Buffer WB	15-25 °C	6 ml (add ethanol before first use)	15 ml (add ethanol before first use)
Elution Buffer EB	15-25 °C	15 ml	15 ml
Spin columns AC	15-25 °C	20	50
Collection tubes (2 ml)	15-25 °C	20	50

Before first use, add the volume of absolute ethanol indicated on the Wash Buffer WB label and tick the box on the label.

Description

During apoptosis, chromatin DNA is cleaved between nucleosomes and eventually forms DNA fragments that are integer multiples of 200 bp. After extraction, these fragments give a ladder-like pattern on electrophoresis after ethidium bromide staining and are called a DNA ladder. Blood cells and cultured cells are lysed in Lysis/Binding Buffer CB; the released DNA fragments bind selectively to the silica membrane of the spin column under high chaotropic salt conditions. A series of rapid wash and centrifugation steps removes salts, cell metabolites, proteins and other impurities, and the DNA ladder fragments are finally eluted from the silica membrane with a low-salt elution buffer.

Features

No toxic phenol or other reagents and no ethanol precipitation step are required.

The dedicated lysis/binding buffer formulation lyses cells efficiently; no expensive proteinase K treatment is needed, which reduces cost and speeds up processing.

Fast and simple: a single sample is usually processed within 10 minutes.

Notes before starting

Note: All centrifugation steps are carried out at room temperature at 13,000 rpm in a conventional benchtop centrifuge such as an Eppendorf 5415C or similar.

Note: Preheat the water bath to 70 °C before starting.

Note: Lysis/Binding Buffer CB contains irritants. Wear latex gloves and avoid contact with skin, eyes and clothing. Rinse affected areas with plenty of water or saline.

Note: Typical DNA yield is 10-20 μg from 2 × 10^6 cultured cells and 3-6 μg from 200 μl of human whole blood.

Note: A typical loading amount for electrophoresis is 2-3 μg of purified DNA. If the apoptosis rate is low, only genomic DNA may be seen and no DNA ladder; try increasing the loading amount.

❖ Procedure

Before first use, add the indicated amount of absolute ethanol to Wash Buffer WB.

1. Take 2 × 10^6 cells (suspension cells or cultured cells resuspended in 200 μl PBS) or 200 μl of whole blood (about 2 × 10^6 cells), add 200 μl of Lysis/Binding Buffer CB and vortex immediately to mix thoroughly.

Optional: if residual RNA interferes with observation of the apoptotic DNA ladder, add 20 μl of RNase A (25 mg/ml) before adding buffer CB, vortex to mix and leave at room temperature for 5-10 minutes.

The maximum starting volume of cells or whole blood is 300 μl. If the starting volume is between 200 μl and 300 μl, scale up the reagents used in the following steps proportionally.

2. Leave at room temperature (15-20 °C) for 10 minutes.

3. Add 100 μl of isopropanol and vortex immediately to mix thoroughly; a flocculent precipitate may appear.

Thorough mixing is very important; insufficient mixing severely reduces yield. If the sample is viscous and difficult to mix, vortex for 15 seconds.

4. Load the mixture from the previous step (including any precipitate) onto spin column AC placed in a collection tube; centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

5. Add 600 μl of Wash Buffer WB (check that ethanol has been added), centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

6. Add 600 μl of Wash Buffer WB, centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

7. Return column AC to the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can reduce elution efficiency and affect downstream reactions.

8. Transfer column AC to a clean centrifuge tube. Apply 100 μl of Elution Buffer EB (prewarmed in a 65-70 °C water bath) to the centre of the membrane, leave at room temperature for 3-5 minutes and centrifuge at 12,000 rpm for 1 minute. Reload the eluate onto the column, leave at room temperature for 2 minutes and centrifuge at 12,000 rpm for 1 minute.

A larger elution volume gives higher elution efficiency. If a higher DNA concentration is required, the elution volume may be reduced, but not below 50 μl; too small a volume reduces DNA elution efficiency and yield.

9. The DNA can be used directly or stored at -20 °C for no more than 14 days.

10. Electrophorese approximately 2-3 μg of purified DNA (note that 200 μl of whole blood typically yields only 3-6 μg).

Troubleshooting

Problem	Comments and suggestions

No DNA ladder bands, only the genomic DNA band of non-apoptotic cells	*Cells did not undergo apoptosis or the apoptosis rate is too low. Suggestion: increase the concentration of the apoptosis inducer or prolong the induction time.
No DNA ladder bands and no genomic DNA band either	*The amount of isolated DNA is too low. Suggestion: increase the starting amount of cells and scale up the reagents proportionally; make sure step 7 is performed so that residual ethanol does not reduce elution efficiency. /*The sample itself contains little DNA (e.g. whole blood) and the loading amount is too low. Suggestion: load more of the purified DNA on the gel.
DNA is smeared, no ladder	*Late-stage apoptosis with non-specific DNA cleavage. Suggestion: extract the DNA ladder at an earlier stage of apoptosis (or perform a time-course with several time points).
High background, DNA ladder weak or indistinct	*Too much RNA contamination interferes with observation. Suggestion: add DNase-free RNase to the eluted purified DNA to a final concentration of 2 μg/ml and leave at room temperature (15-20 °C) for 20 minutes to degrade contaminating RNA. Normal cells contain more RNA, so when the proportion of apoptotic cells is too low, more RNA tends to remain; high residual RNA often indicates a low apoptotic fraction, and the induction conditions may be adjusted accordingly.

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