GENE CREATE

# Animal DNA pull-down Kit

## Catalog#JKR23006A

### Instruction Manual (For Two Groups)

Sufficient reagents for 6T assays per kit.
Store at -20& 4°C

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# 1.Experimental Principle

DNA pull-down technology is a powerful tool for studying DNA-protein interactions in vitro. This technique involves designing specific DNA probes targeting the region of interest, labeling them with desthiobiotin, and then allowing the labeled probes to bind to streptavidin conjugated to magnetic beads. After incubation with total protein extract, proteins that interact specifically with the DNA probes form bead-DNA probe-protein complexes. Non-specifically bound proteins are washed away, and the target DNA probe-protein complexes are eluted. Finally, protein types are identified via Western Blot or mass spectrometry (MS). A schematic diagram is shown in 1.1:

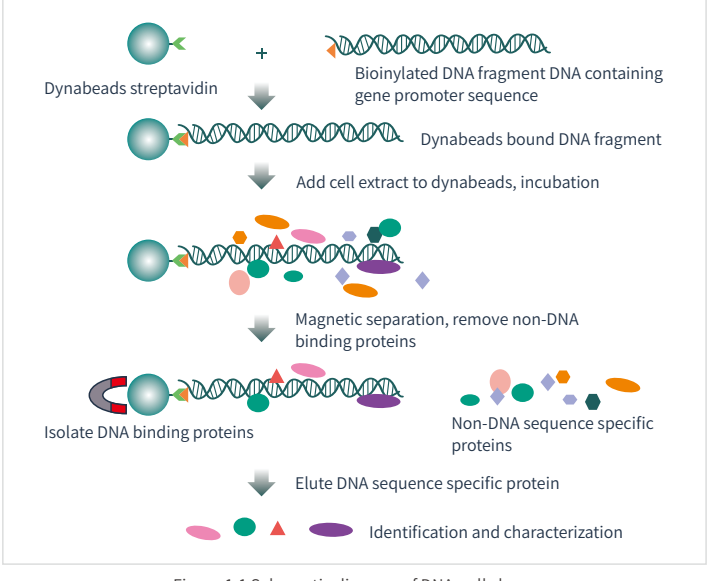
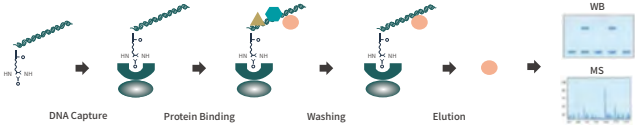


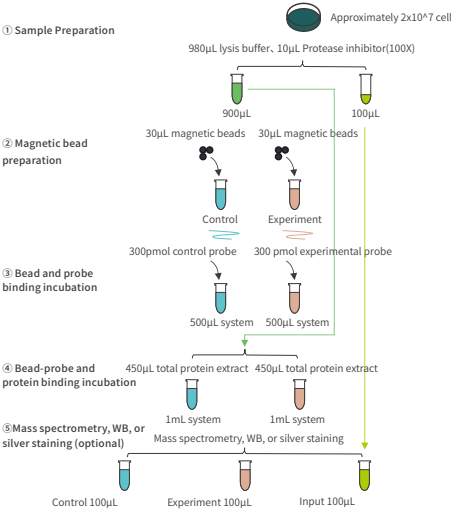

Figure 1.1 Schematic diagram of DNA pull-down

# 2.Experimental Procedure

## 2.1 Experimental Flowchart




## 2.2 Control Setup Flowchart



## 3.Kit Components

| Component | Volume(6T) | Storage Condition |
|---|---|---|
| Lysis buffer | 9mL | 4°C |
| Protease inhibitor (100 X) | 35μL | -20°C |
| Nucleic dilution buffer | 30mL | 4°C |
| Protein dilution buffer | 45mL | 4°C |
| Nucleic-Acid Compatible Streptavidin Magnetic Beads | 200μL | 4°C |
| Elution buffer | 800μL | Store at 4°C protected from light |

**Special note: 6T corresponds to 6 single-group (1 experimental group or 1 control group) immunoprecipitation experiments; the subsequent procedure includes one experimental group and one control group, consuming 2T of reagent.**

## 4.Operating Steps

### 4.1 Total Protein Extraction

#### 4.1.1 Cell Samples

① Washing: Wash the sample (approximately 2×10^7 cells) twice with 1 mL of pre-cooled PBS, and remove as much PBS as possible after the last wash;
② Lysis: Add 980 μL Lysis buffer and 10 μL Protease inhibitor (100X) according to cell count, fully lyse on ice for 30 min, vortex every 5 min for 10s each time;
③ Sonicate for 5 min using an ultrasonic cell disruptor at 20% power, with 3s on and 3s off, in an ice bath;
④ Centrifugation: 4°C, 12000 rpm, 10 min, collect the supernatant.

#### 4.1.2Tissue Samples

① Grinding: Take fresh or cryopreserved tissue (approx. 0.3g), place in a sterilized pre-cooled mortar, and grind to a powder using liquid nitrogen;
② Take 980 μL of Lysis buffer and 10 μL of Protease inhibitor (100X), mix well to

prepare the lysis solution. Pipette 800 μL of the lysis solution into a mortar, continue grinding on ice for 5-10 min until the sample becomes a fine homogeneous slurry, transfer to a new EP tube. Then add the remaining 190 μL of lysis solution to the mortar to collect residual sample, and similarly transfer it to the same EP tube;

③ Allow the EP tube containing the sample slurry to fully lyse on ice for 30 min, vortexing once every 5 min for 10 sec each time;

④ Sonicate using an ultrasonic cell disruptor for 8-10 min at 20% power, with 3 sec of sonication and 3 sec of interval, performing ice-bath sonication;

⑤ Centrifugation: 4°C, 12000 rpm, 10 min, collect the supernatant, then add Lysis buffer to the supernatant to bring the total volume to 1 mL, mix well.

Note: Perform the entire protein extraction process on ice to reduce protein degradation caused by high temperature; avoid bubble formation during sonication to minimize protein degradation. Store the total protein after lysis at -20°C.

## 4.2 Magnetic bead preparation and washing

① Take the Nucleic-Acid Compatible Streptavidin Magnetic Beads from the 4°C refrigerator, invert several times to mix the bead storage solution, pipette 30μL each into two 1.5 mL Eppendorf tubes, labeled as control group and experimental group, place on a magnetic stand for 1min to separate the beads, and discard the supernatant;

② Add 500 μL of Nucleic dilution buffer to both the control and experimental groups, resuspend the beads, place on a magnetic stand for 1min, and discard the supernatant. Repeat this step three times.

## 4.3 Bead Binding to DNA

① Add 100-300 pmol of biotin-labeled DNA probe to the experimental tube; add an equal amount of non-biotin-labeled DNA or none to the control tube. Bring the volume to 500 μL with Nucleic dilution buffer, and incubate at room temperature on a silent mixer for 2 hours;

② Remove the control and experimental tubes from the silent mixer, place them on a magnetic stand for 1 minute, and discard the supernatant;

③ Add 500 μL of Nucleic dilution buffer to each control and experimental tube, resuspend the magnetic beads, place on a magnetic stand for 1 minute, and discard the supernatant. Repeat this step 3 times.

### 4.4 DNA-Bead Binding Protein

① Add 450 μL of extracted protein to each control and experimental tube, supplement the volume to 1 mL with Protein dilution buffer, and incubate overnight (approximately 16 h) at 4°C on a silent mixer, reserving 100 μL of lysate here as the Input group;

② Remove the control and experimental tubes from the silent mixer, place them on a magnetic stand for 1 min, and discard the supernatant;

③ Add 1 mL of Protein dilution buffer, resuspend the beads, place on the magnetic stand for 1 min, discard the supernatant, and repeat this step 5 times.

### 4.5 Elution of Complexes

① Add 100 μL of Elution buffer to both the control and experimental tubes, mix well, then heat in a boiling water bath for 8-10 min. Place on a magnetic rack and let stand for 2 min, transfer the supernatant to new EP tubes—this is the pull-down product, labeled as the control group and experimental group. Add 20 μL of 6X Loading buffer to each of the two tubes, and heat in a boiling water bath for 8-10 min;

② Reserve 100 μL of lysate from the Input group, also add 20 μL of 6X Loading buffer, and heat in a boiling water bath for 8-10 min;

③ Store the control group, experimental group, and Input at -20°C for later use. Subsequent steps include silver staining, mass spectrometry identification, or WB detection.

## 5. Frequently Asked Questions

**Q: After pull-down and silver staining validation, no desired target band is observed?**

① The sample is degraded by proteases; the corresponding strategy is to add protease inhibitors, perform all operations on ice below 4°C, and avoid repeated freeze-thaw cycles.

② Insufficient biotin-labeled DNA added; the amount of biotin-labeled DNA can be increased.

③ The salt-alkalinity of the lysis buffer is too high; a lysis buffer with lower salt-

④ alkalinity should be used.

Insufficient cell lysis buffer added; the amount of cell lysis buffer can be increased.

Silver staining is limited by the sensitivity of the experiment itself; even if the target protein is enriched, it may not be visible in silver staining. Silver staining primarily serves as a quality control measure to assess whether the entire experimental procedure is abnormal, such as the total protein amount after enrichment. It cannot determine the final mass spectrometry identification results, and it is generally recommended to rely on the mass spectrometry results。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