

Kits for Studying Gene Regulation

Gene expression studies often require analysis of the steps involved in transcription. Choose from several Roche Molecular Biochemicals kits for RNA and promoter analysis, including the DIG Gel Shift Kit, DNA-binding Protein Purification Kit, RNase Protection Kit, and Northern ELISA.

DIG Gel Shift Kit

For identification of sequence-specific DNA-binding proteins in cell extracts

Description and use

Use this kit's ready-to-use reagents for gel shift reactions, including DNA labeling, DNA/protein binding, and chemiluminescent detection (Table 21; Figures 44, 45).

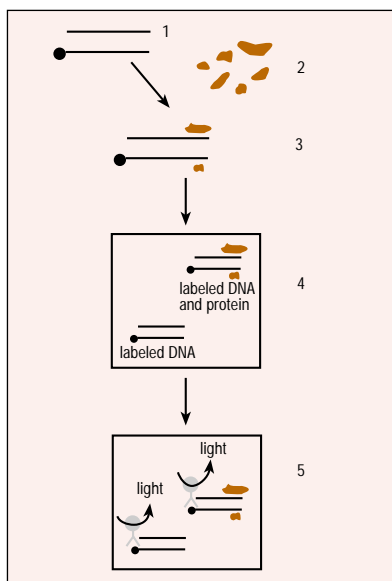


Figure 45. Principle of the DIG Gel Shift Kit.

DNA oligonucleotides (step 1) are labeled with DIG (•) and added to extracts containing DNA-binding proteins (step 2). The appropriate DNA-binding proteins interact specifically with the oligonucleotide (step 3). The resulting complexes are electrophoresed on native polyacrylamide gels. Free DNA and DNA:protein complexes migrate at different rates. DNA migration is "shifted" or "retarded" by binding of proteins (step 4). After transfer to nylon membranes, the DIG-labeled oligonucleotides are detected using a chemiluminescent reaction (step 5).

Advantages

- **Avoid extensive optimization of gel shift procedures**
Save the time and effort typically devoted to establishing gel shift procedures in the lab. Instead, choose a kit containing all the required reaction components, controls, and an optimized protocol.
- **Eliminate radioactivity by labeling with digoxigenin-11-dUTP (DIG)**
Eliminate the long exposure times, record-keeping, and safety hazards required by radioisotopes. Also, store the stable DIG-labeled oligonucleotide probes for reuse in multiple gel shift assays.
- **Use several types of DNA**
Label synthetic oligonucleotides, as well as double-stranded DNA fragments, using a versatile 3'-end-labeling reaction catalyzed by terminal transferase.
- **Prevent large, nonspecific DNA/protein complexes**
Add the provided poly d(A-T) and poly d(I-C) to the extract to prevent nonspecific protein binding.

+	+	+
-	+	+
-	-	+

DIG-labeled Oligo
Oct2A
competitor Oligo

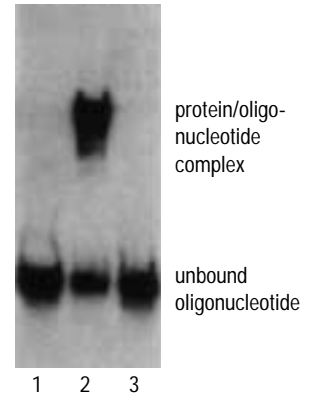


Figure 44. Detection of DNA-binding proteins with the DIG Gel Shift Kit.

1. Labeling buffer (5x)
2. CoCl₂
3. DIG-11-ddUTP
4. Terminal transferase
5. Binding buffer (5x)
6. Unlabeled control oligonucleotide
7. DIG-labeled control oligonucleotide
8. control factor Oct2A
9. poly [d(I-C)]
10. poly [d(A-T)]
11. poly-L-lysine
12. loading buffer without bromophenol blue
13. loading buffer with bromophenol blue
14. Anti-DIG-AP, Fab fragments
15. CSPD chemiluminescent AP substrate
16. Blocking reagent

Table 21. Contents of the DIG Gel Shift Kit.

Product	Cat. No.	Pack Size
DIG Gel Shift Kit	1 635 352	1 kit (20 labeling and 200 gel shift reactions)

1. Streptavidin-magnetic particles with tethered oligonucleotide (46-mer)
2. Buffer TEN 2000
3. Oligo ligation buffer (5x)
4. PEG 6000
5. KCl
6. T4 DNA ligase
7. Protein binding buffer
8. Poly[d(I-C)]
9. Poly-L-lysine
10. Double-stranded control oligonucleotide
11. Control crude extract (contains Oct2A DNA-binding protein)
12. Dialysis buffer

Table 22. Contents of the DNA-binding Protein Purification Kit.

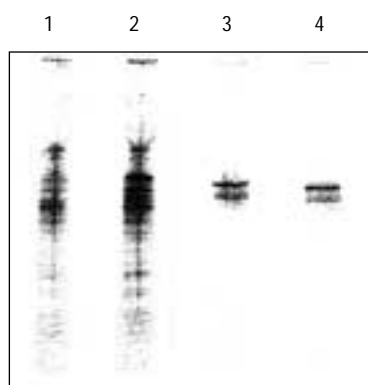


Figure 47. Typical results obtained with the DNA-binding Protein Purification Kit. Crude *E. coli* extracts containing transcription factor Oct2A were purified using an oligonucleotide with an Oct2A-specific binding site. Bound proteins were separated by SDS-PAGE and visualized by silver staining.

Lane 1: *E. coli* extract without Oct2A factor (400 ng total protein)

Lane 2: *E. coli* extract containing 5% Oct2A factor

Lane 3: Oct2A purified from crude *E. coli* extract

Lane 4: Oct2A purified according to a standard reference method.

DNA-binding Protein Purification Kit

For affinity purification of sequence-specific DNA-binding proteins from cell extracts

Description and use

Use this kit for the one-step affinity-purification of proteins binding to a known DNA target sequence (Table 22, Figure 46).

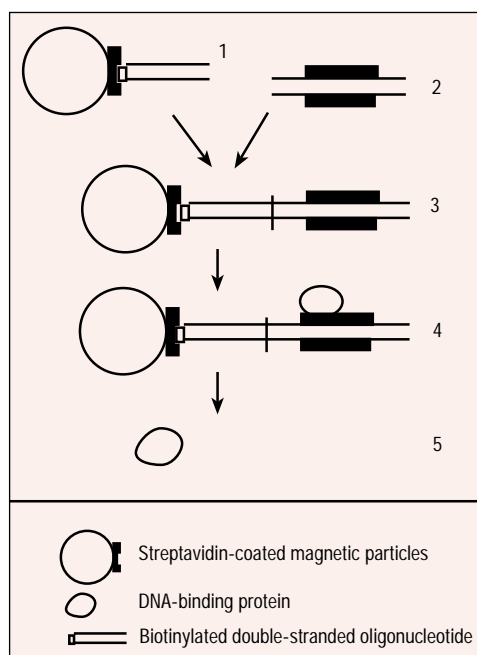


Figure 46. Principle of the DNA-binding Protein Purification Kit. The kit's 3'-biotinylated double-stranded 16-mer oligonucleotide, which is tethered to streptavidin-coated magnetic particles (step 1), is ligated to 30–50-mers containing the sequence for protein binding (step 2). This forms concatamers with high binding capacities (step 3). The DNA concatamers are added to protein extracts under conditions where specific protein binding will occur (step 4). Following magnetic separation and washing, the DNA-binding proteins are eluted in high ionic strength buffer (step 5).

Advantages

- **Avoid affinity chromatography**
Use straight-forward procedures, such as ligation and magnetic bead technology.
- **Save the time normally required to establish procedures for purifying DNA-binding proteins**
Use this kit's optimized reagents, protocols, and controls to obtain purified protein (Figure 47) in less than two hours.

Product	Cat. No.	Pack Size
DNA-binding Protein Purification Kit	1 835 513	1 kit (purification of 2–40 µg DNA-binding protein)
Also Available	Cat. No.	Pack Size
Magnetic Particle Separator	1 641 794	1 separator

RNase Protection Kit

For detection and quantification of RNA

Description and use

Use this kit's ready-to-use solutions for RNase protection assays (Table 23, Figure 48). RNase protection assays are useful for analyzing gene and mRNA structure and quantities or for mapping transcription termination sites.

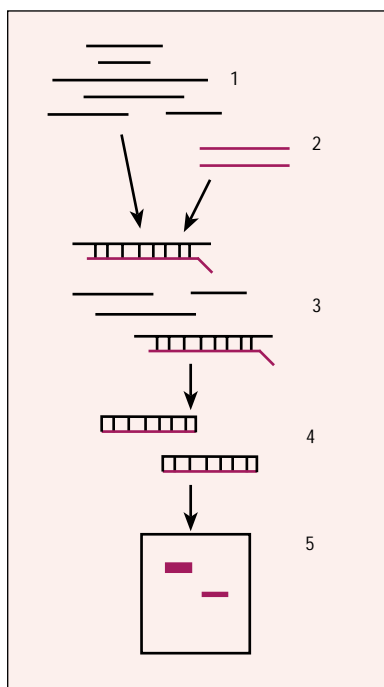


Figure 48. Principle of the RNase Protection Kit.

An RNA sample (step 1) is combined with an excess of labeled antisense RNA probes (step 2) generated by SP6, T7, or T3 RNA transcription. The RNA probes (in red) hybridize to these complementary sequences (step 3). RNase A and RNase T1 degrade all RNA that is not in duplex form (step 4). The resulting duplexes are electrophoresed in polyacrylamide gels (step 5), where they can be analyzed for size or quantified for amount of radioactivity.

1. Hybridization buffer
2. RNase digestion buffer
3. Control RNA
4. RNase A
5. RNase T1
6. Proteinase K
7. SDS (20%)
8. Yeast tRNA
9. Control DNA
10. Loading buffer

Table 23. Contents of the RNase Protection Kit.

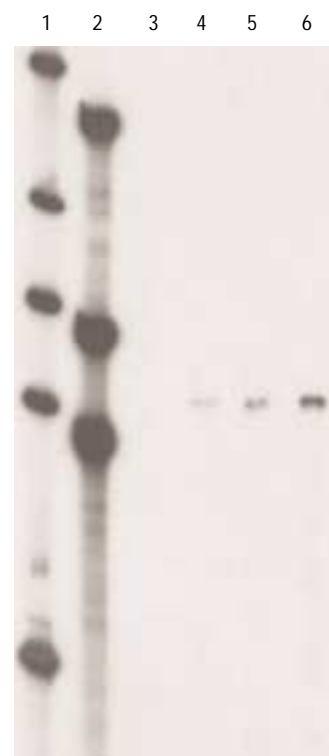


Figure 49. Detection of LDL receptor mRNA using the RNase Protection Kit.

LDL receptor mRNA was detected in total RNA isolated from human liver. Lanes 1 and 2 contain molecular weight markers; Lanes 3, 4, 5, and 6 contain 0, 5, 10, and 25 µg of RNA, respectively.

Advantages

- **Choose nonradioactive or radioactive labels**
Perform the standard radioactive assay or an optimized nonradioactive assay using RNA probes labeled with digoxigenin-11-UTP.
- **Simplify the establishment of RNase protection assay procedures**
The required reagents, controls, and optimized protocol provided in this kit eliminate the need to waste time and effort developing new RNase protection assay procedures. Obtain reliable results without extensive optimization (Figure 49).

Product	Cat. No.	Pack Size
RNase Protection Kit	1 427 580	1 kit (100 standard reactions and 5 control reactions)
Also Available	Cat. No.	Pack Size
RNase A	109 142	25 mg
	109 169	100 mg
RNase T1	109 193	100,000 units (1 ml)
	109 207	500,000 units (1 ml)

1. mRNA labeling reagent
2. Hybridization buffer
3. Washing buffer (10x)
4. Conjugate dilution buffer
5. Anti-digoxigenin-POD
6. TMB substrate solution
7. Stop reagent
8. Positive control probe, DIG-labeled
9. Negative control probe, DIG-labeled (CAT)
10. Streptavidin-coated microtiter plate, with 12 modules of 8 wells
11. Self-adhesive plate cover foils

Table 24. Contents of the Northern ELISA.

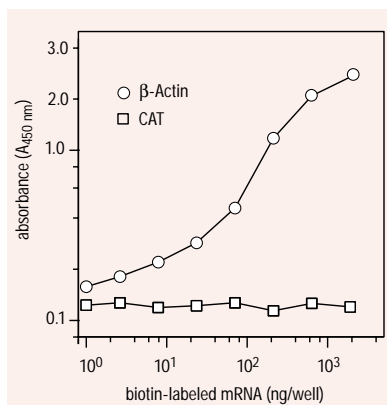


Figure 51. Detection of β -actin mRNA in the total population of poly(A)⁺ RNAs isolated from cell line K562. Increasing amounts of biotinylated poly(A)⁺ RNA were bound to streptavidin-coated microtiter plate wells and hybridized with excess amounts of DIG-labeled DNA probes to β -actin and CAT (negative control). Note that quantification of β -actin mRNA can be achieved.

Northern ELISA (chromogenic)

For detection and quantification of mRNA in microtiter plates

Description and use

Use this kit's ready-to-use reagents to detect and quantify poly(A)⁺ RNA from eukaryotic cells in a microtiter plate format (Table 24, Figure 50). The kit is useful for simultaneous analysis of several different RNA preparations or probes.

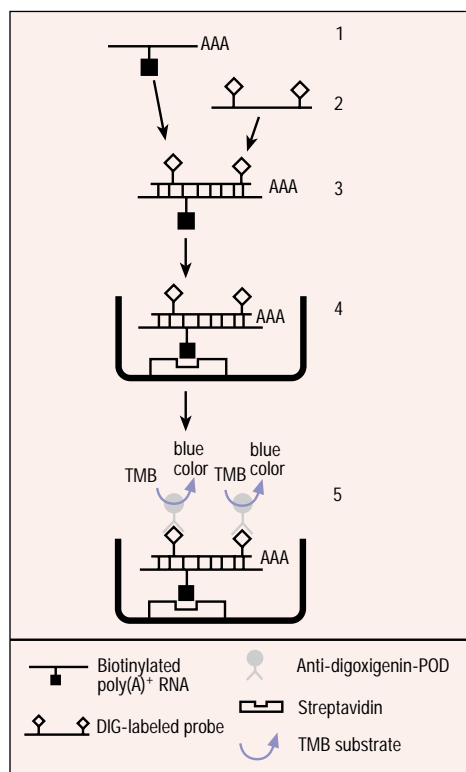


Figure 50. Test procedure of the Northern ELISA. Poly(A)⁺ RNA is isolated from cells or tissues and is chemically biotinylated (step 1). Separately, DIG-labeled DNA probes are prepared (step 2). The probe and sample are combined and hybridized (step 3). The hybrids are captured in a streptavidin-coated microtiter plate (step 4) and detected using Anti-DIG antibodies coupled to horseradish peroxidase. Addition of TMB peroxidase substrate produces a blue color (step 5).

Advantages

- **Achieve sensitive, quantitative results**
Accurately quantify mRNA with an ELISA reader (Figure 51), and achieve results that are up to 10 times more sensitive than those of classical Northern blotting.
- **Increase convenience**
The convenient ELISA format simplifies the handling of a large number of samples, and no hazardous radioisotopes are required.
- **See your results sooner**
The entire procedure – labeling of isolated mRNA to detection of specific transcripts – takes less than one day and requires less than two hours of hands-on time.

Product	Cat. No.	Pack Size
Northern ELISA (chromogenic)	1 758 403	1 kit (96 reactions)