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ProductInformation

AP-1 Consensus Oligonucleotide

Product Code **A 9590**
Storage Temperature –20 °C

The transcription factor designated AP-1 is a protein complex mixture[1] of Jun family (c-Jun, JunB, and JunD), homodimers and heterodimers with the Fos family (c-Fos, FosB, Fra-1, and Fra-2),[2,3,4] or with Fos-related proteins, CREB or ATF-2.[5] The dimerization is mediated by a carboxy-terminal coil structure, termed the leucine zipper, and is necessary for DNA binding to a palindromic sequence known as the TPA-responsive element (TRE) or AP-1 consensus site, existing in many gene enhancers.[6] The AP-1 complex is regulated by *de novo* synthesis as well as by post-translational modifications such as phosphorylation and dephosphorylation that may influence their binding combinations.[1] The AP-1 complex regulates gene expression either positively or negatively, depending on the interaction with different Fos/Jun or Jun/Jun dimers. Domain mapping experiments indicate that c-Jun interacts with the conserved C-terminus of TATA-binding protein and TFIIB *in vitro.* The AP-1 transcriptional complex has been implicated in a number of biological processes like cell cycle progression, differentiation, and transformation. c-Jun has also been linked to apoptosis.[7]

AP-1 consensus oligonucleotide is supplied as dry, blunt-ended double-stranded synthetic oligonucleotide. When reconstituted in 20 µl of water, the final concentration is 1.75 pmole/µl (1.75 µM) and is ready to be end labeled with polynucleotide kinase. This consensus oligonucleotide is designed to be used in mobility shift assays screening for the presence of AP-1 transcription factor in various extracts.

The AP-1 consensus oligonucleotide is sufficient for at least 500 tests when used according to the following procedure.

Reagent

- AP-1 consensus oligonucleotide, 1 vial
  Product Code A 9590
  5'- CGC TTG ATG ACT CAG CCG GAA -3'
  3'-GCG AAC TAC TGA GTC GGC CTT –5'

Reagents and Equipment Required But Not Provided

(Product Codes have been given where appropriate)

Reagents for Non Denaturing Polyacrylamide Gel Preparation

- Molecular biology grade water, Product Code W 4502
- Desired buffer for electrophoresis
  - 10x TAE (Tris-acetate-EDTA) buffer, Product Codes T 9650, T 8280, or T 4038
    or
  - 10x TBE (Tris-borate-EDTA) buffer, Product Code T 4415
    or
  - Trizma® base, Product Code T 8524, glycine, Product Code G 4392 and EDTA, Product Code E 5134
- Acrylamide/bis-acrylamide (19:1), 40% solution, Product Code A 2917
- Ammonium persulfate, Product Codes A 9164 or A 3426
- TEMED, Product Code T 7024
- Gel electrophoresis equipment

Reagents for DNA Consensus Oligonucleotide Labeling

- γ-^{32}P-ATP (3000 Ci/mmol at 10 mCi/ml)
- 0.5 M EDTA, Product Code E 7889
- 100x TE (Tris-EDTA) buffer, Product Code T 9285
- 1x TE buffer (Dilute 100x TE buffer 100-fold with water)
- Stop reaction buffer (Add 10 µl of 0.5 M EDTA to 890 µl 1X TE buffer)
- 10x T4 polynucleotide kinase buffer without ATP (300 mM Tris-HCl, pH 7.8, 100 mM $MgCl_2$, 10 mM dithiothreitol, 1 mg/ml BSA, 12 µg/µl micrococcal-nuclease-treated salmon DNA)
- Polynucleotide kinase (5-10 units/µl), Product Code P 4390
- Molecular biology grade water
- Oligonucleotide (double stranded) with dephosphorylated 5'-end.
- Sephadex® G-25 mini column

Reagents for Mobility Shift Assay

- 5% polyacrylamide gel from Procedure Section A
- Mobility shift optimization kit, Product Code SHIFT-1
- 5x Binding Buffer A (60 mM HEPES, 20 mM Tris-HCl, pH 8.0, 300 mM KCl, 5 mM EDTA, 5 mM EGTA, and 60% glycerol)
- γ-^{32}P labeled AP-1 from Procedure Section B
- HeLa cell nuclear extract
- Non-relevant consensus DNA, SP-1, Product Code S 0431 or any appropriate non-relevant consensus DNA
- 10x Loading buffer (250 mM Tris-HCl, pH 7.5, 40% glycerol, and 0.2% bromophenol blue)
- 1% BSA, prepared from Product Code A 2153 or equivalent
- 100 mM Dithiothreitol (DTT), prepared from Product Code D 9779
- Poly(dI-dC)•poly(dI-dC), Product Code P 4929 (Dissolve in molecular biology grade water to prepare a 1 µg/µl solution)
- Molecular biology grade water
- Blotting paper, Product Codes P 6664, P 6914, or P 9039

Precautions and Disclaimer

The AP-1 consensus oligonucleotide is for laboratory use only, not for drug, household, or other uses. When radioactive tracers are used, standard procedures for safely handling radioactive materials should be followed.

Storage

Store at –20°C. Repeated freezing and thawing is **not** recommended

Preparation Instructions

Reconstitute AP-1 consensus oligonucleotide with 20 µl water to give a final concentration of 1.75 pmole/µl.

Procedure

A. Non Denaturing Polyacrylamide Gel Preparation

There are several possibilities for running the nondenaturing gel which include different acrylamide concentrations and different buffers. Typical gels used in this assay are in the range of 4% to 6% acrylamide.

Many gel buffers have been successfully used for mobility shift assays. The stability of DNA-protein complexes can be strongly affected by the choice of buffer, so it is worth trying different systems for the protein of interest. The most common buffers used are Tris-acetate-EDTA (TAE), Tris-glycine-EDTA (TGE), and Tris-borate-EDTA (TBE). The electrophoresis buffers are typically used at 0.5x, 1.0x, or 2.0x concentrations. The gel running buffer should match the buffer used to prepare the gel. Table 1 may be used to prepare a 5% acrylamide/bis-acrylamide gel; the buffer may be changed in order to optimize of the mobility shift assay.

See Reagents and Equipment Required but Not Provided section for list of required reagents.

Table 1.
Preparation of 5% nondenaturing gel mix

	0.5x Buffer	1x Buffer
40% Solution acrylamide/bis-acrylamide (19:1)	12.5 ml	12.5 ml
10x Buffer	5 ml	10 ml
Water	81.5 ml	76.5 ml
10% Ammonium persulfate	1 ml	1 ml
TEMED	125 µl	125 µl
Total volume	100 ml	100 ml

10x buffer used may be TAE, TBE, or TGE.

Formulation for 10x TGE (Tris-glycine-EDTA) buffer:

30.28 g	Trizma base	(0.25 M)
142.7 g	Glycine	(1.9 M)
3.92 g	EDTA	(10 mM)

Add water to a final volume of 1 liter and adjust the pH to approximately 8.3.

Cast the gel as per standard protocols for use in the mobility shift assay.

B. DNA Consensus Oligonucleotide Labeling

Use the following procedure for γ-^{32}P 5'-end labeling of the AP-1 consensus oligonucleotide.

See Reagents and Equipment Required but Not Provided section for list of required reagents.

1. Phosphorylation Reaction

   a. Assemble the following reaction in a sterile microcentrifuge tube in an ice bath:

      2 µl oligonucleotide (dephosphorylated) (1.75 pmole/µl)

      or

      2 µl AP-1 consensus oligonucleotide (1.75 pmole/µl)

      1 µl Polynucleotide kinase 10x buffer

      1 µl Polynucleotide kinase

      1-3 µl γ-^{32}P ATP

      Notes: Use 1 µl γ-^{32}P ATP within the first week of receipt and 2-3 µl within the second week.

      Add γ-^{32}P ATP last to the reaction mixture.

      up to 10 µl molecular biology grade water

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      10 µl Total volume

   b. Incubate at 37 °C for 30 minutes.

   c. Stop the reaction by adding 90 µl of stop reaction buffer.

2. Removal of Unincorporated Label by Sephadex G-25 Chromatography

   a. Follow procedure according to manufacturer's instructions. Expected incorporation is 40-70%.

   b. Store the radiolabeled reagent at –20 °C. The material is stable for a minimum of 2 weeks at –20 °C.

C. Mobility Shift Assay

See Reagents and Equipment Required but Not Provided section for list of required reagents.

1. Remove the comb from the previously prepared 5% gel and set the gel in an electrophoresis unit.

2. Pre-run the gel without sample for 30-60 minutes at 10 V/cm.

3. Thaw the kit components if using the mobility shift optimization kit, Product Code SHIFT-1.

4. Prepare 3 marked tubes for AP-1 labeled consensus DNA:

   Tube 1 Basic reaction to observe shifted bands

   Tube 2 Competition with unlabeled AP-1 to observe the interference or elimination of the specific shifted band

   Tube 3 Competition with unlabeled nonrelevant DNA to confirm there is no interference with the specific shifted band

5. Combine the reagents according to the following table:

Reagents	**Tube Number**		
	1	2	3
5x binding buffer A	6 µl	6 µl	6 µl
100 mM DTT	0.5 µl	0.5 µl	0.5 µl
1% BSA	2 µl	2 µl	2 µl
Poly(dI-dC)• poly(dI-dC), 1 µg/µl	2 µl	2 µl	2 µl
HeLa cell nuclear extract, 5-10 mg/ml	2 µl	2 µl	2 µl
Unlabeled AP-1 consensus oligonucleotide, 1.75 pmole/µl	----	2 µl	----
Unlabeled nonrelevant probe, 1.75 pmole/µl	----	----	2 µl
Water	15.5 µl	13.5 :l	13.5 µl
Incubate 10 minutes at room temperature, then add:			
γ-^{32}P labeled AP-1 consensus oligonucleotide	2 µl	2 µl	2 µl

Mix each tube gently by tapping the bottom of the tube with a finger. Avoid introducing bubbles in the mix. Spin the tubes in a microcentrifuge for few seconds. Incubate the reaction tubes for 20 minutes at room temperature.

6. After incubation, add approximately 3 µl of loading buffer to each tube.

7. Stop the pre-run of the gel started in Step 2.

8. Load the contents of each tube into separate wells on the gel. There is no stacking gel in the system, so precise loading with minimum mixing of the buffer is necessary to obtain sharp bands. Washing the wells with running buffer prior to loading the samples is recommended; empty the wells by inverting the gel on blotting paper. Allow the sample (~30 µl) to fall along one side of the well to prevent dilution of the sample and avoid bubbles in the well.

9. Carefully add running buffer to the wells with a pipette and then add buffer to the upper electrophoresis chamber.

10. Electrophorese at 10 V/cm to give a clear separation of the free labeled DNA consensus and the DNA-protein complexes. Stop the gel run when the dye is 2/3 of the way through the gel.

11. Remove the gel from the box and carefully remove the side spacers.

12. Using a spatula, slowly separate the glass plates apart, allowing air to enter between the gel and the upper glass plate.

13. Lay the lower glass with the gel facing up on a hard surface. Place three sheets of blotting paper cut to size on top of the gel.

14. Peel the filter paper with the gel attached to it from the plate.

15. Cover the gel with a nylon membrane and dry under vacuum (~1.5 hours at 80 °C).

16. Autoradiograph the dried filter overnight at room temperature (or overnight at –70 °C, with an intensifying screen).

Results

γ-^{32}P labeled AP-1 consensus oligonucleotide forms a shifted, specific band when tested with HeLa cell nuclear extract as the DNA binding protein. Unlabeled AP-1 consensus oligonucleotide competes with the shifted band and reduces or eliminates it. Nonspecific oligonucleotide does not interfere with the shifted band.

References

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