

## Product Contents

### rhSP1 (human):

<b>Part No.</b>	<b>Size</b>
E639A	15µg per vial

**Description:** The SP1 transcriptional control sequence is found in a large number of viral and cellular promoters, including the HIV long terminal repeat. The SP1 transcription factor contains contiguous zinc finger motifs that provide specific DNA binding activity. SP1 factor interacts with other coactivator proteins that can modulate transcriptional activity. The SP1 recombinant protein is expressed from a human cDNA clone (1) in Sf9 cells using a baculovirus expression system.

The sequence of the oligonucleotide used in the purification is:

5'...GCTCGCCCGCCCGATCGAAT...3'  
3'...CGAGCGGGCGGGCTAGCTTA...5'

rhSP1 (Cat.# E6391) migrates with an apparently lower molecular weight than the protein expressed in mammalian cells. When rhSP1 protein preparations are separated using 4–20% SDS PAGE and visualized by Coomassie® stain, protein species with apparent molecular weights of approximately 82kDa and 57kDa were observed. Proteins produced in Sf9 cells are not expected to undergo the same style of glycosylation as that in mammalian cells.

Please note that Promega's rhSP1 has not been used in transcription activation assays. For in vitro transcription protocols using this type of SP1 preparation, see reference 2.

**Storage Buffer:** 5µM ZnSO<sub>4</sub>, 50mM KCl, 2mM DTT, 12mM HEPES-KOH (pH 7.5 at 22°C), 6mM MgCl<sub>2</sub>, 0.1% NP40 and 50% glycerol.

**Storage Temperature:** Store at –70°C. Always avoid multiple freeze-thaw cycles or exposure to frequent temperature changes. These fluctuations can greatly alter product stability. When stored and handled properly, the performance of this product is guaranteed for at least six months from the date of purchase.

## Quality Control Assays

**Protein Concentration:** See concentration on the product label. Protein concentration is determined by Bradford assay using BSA as a standard.

**Gel Shift Assay Conditions:** Thirty-five femtomoles of labeled SP1 oligonucleotide and 0–300ng of rhSP1 (diluted in a constant volume of 12mM HEPES [pH 7.5], 50mM KCl, 6mM MgCl<sub>2</sub>, 5µM ZnSO<sub>4</sub>, 2mM DTT, 0.1% Nonidet® P-40, 50% glycerol) are incubated at 23°C for 20 minutes in 4% glycerol, 1mM MgCl<sub>2</sub>, 0.5mM EDTA, 0.5mM DTT, 50mM NaCl, 10mM Tris-HCl (pH 7.5), 0.05 mg/ml poly(dI-dC)•(dI-dC). Samples are analyzed on a 6% nondenaturing polyacrylamide gel, which is exposed to X-ray film overnight at –70°C with an intensifying screen. Three hundred nanograms of rhSP1 yields at least a 30% shift of the labeled SP1 oligonucleotide.

**Footprinting Assay Conditions:** Seventy-five femtomoles of an [<sup>32</sup>P] end-labeled 306bp *EcoR I/Hind III* SV40 early promoter fragment and 1µg of rhSP1 (diluted in 12mM HEPES [pH 7.5], 50mM KCl, 5µM ZnSO<sub>4</sub>, 2mM DTT, 0.1% Nonidet® P-40, 6mM MgCl<sub>2</sub>, 50% glycerol) are incubated on ice for 10 minutes in 25mM Tris-HCl (pH 8.0), 50mM KCl, 6.25mM MgCl<sub>2</sub>, 0.5mM EDTA (pH 8.0), 10% glycerol, 0.5mM DTT. To this 50µl assay, 50µl of room temperature 5mM CaCl<sub>2</sub>/10mM MgCl<sub>2</sub> is added, and the reaction is incubated for 1 minute at room temperature. DNase digestion is carried out by the addition of 3µl of diluted RQ1 RNase-Free DNase (5µl RQ1 RNase-Free DNase (Cat.# M6101, 1unit/µl) diluted into 100µl of 10mM Tris-HCl [pH 8.0]), followed by an incubation of 60 seconds at room temperature. Digestion is terminated by the addition of 90µl of 200mM NaCl, 30mM EDTA, 1% SDS, 100µg/ml yeast tRNA and extracted with phenol/chloroform/isoamyl alcohol. Following an ethanol precipitation, DNA fragments are separated on a 6% denaturing polyacrylamide gel. The gel is exposed to X-ray film overnight at –70°C with an intensifying screen. One microgram of rhSP1 yields a strong footprint, which is defined by the presence of 3 major bands in the SP1 footprinting region (see Figure 1).

## References

1. Kadonaga, J.T. *et al.* (1987) Isolation of cDNA encoding transcription factor SP1 and functional analysis of the DNA binding domain. *Cell* **51**, 1079–90.
2. Pugh, B.F. and Tjian, R. (1990) Mechanism of transcriptional activation by SP1: Evidence for coactivators. *Cell* **61**, 1187–97.

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**Promega**

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## I. Gel Shift Assay Protocol

- To prepare the gel shift binding assay, combine the following reagents:

Component	Volume per Gel Shift Reaction
Gel Shift Binding 5X Buffer*	2 $\mu$ l
Labeled SP1 oligonucleotide (35fmol/ $\mu$ l)**	1 $\mu$ l
rhSP1 protein (diluted in SP1 Dilution Buffer)	***
Nuclease-Free Water to a final volume of	10 $\mu$ l

\*The Gel Shift Binding 5X Buffer is available separately as Cat.# E3581.

\*\*Use Promega's SP1 Consensus Oligo (Cat.# E3231) or another suitable oligonucleotide with a putative SP1 binding site. The specific activity of the labeled oligonucleotide should be 5,000–20,000cpm per 10–100fmol.

\*\*\*To see a 30% shift of the SP1 oligonucleotide, use approximately 300ng of rhSP1 protein. In the Quality Control experiments, 3.0 $\mu$ l of 100ng/ $\mu$ l rhSP1 are added.

- Incubate at 23°C for 20 minutes.
- Do not add Gel Loading Buffer to the reactions containing SP1. Instead load 1 $\mu$ l of Gel Loading Buffer into an adjacent lane on the gel as a migration control.
- Load the samples onto a 6% nondenaturing polyacrylamide gel, which has been pre-run in 0.5X TBE buffer for 10 minutes at 300V. The samples contain very little glycerol, so exercise caution when loading the gel.
- After loading the samples, run the gel at room temperature in 0.5X TBE buffer at 250–350V until the bromophenol blue dye is three-fourths of the way down the gel. Maintain a gel temperature of <30°C.
- Expose the gel to X-ray film overnight at –70°C with an intensifying screen.

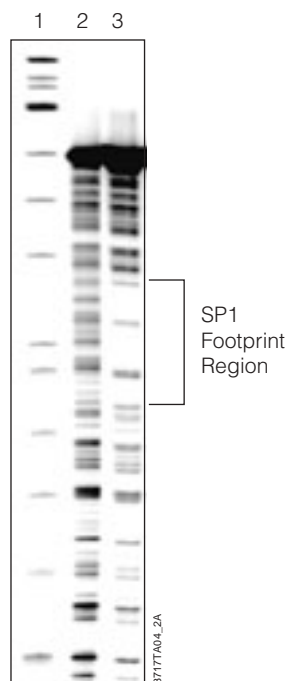
## II. Footprinting Assay Protocol

- Prepare the footprinting assay reaction mix:

Component	Volume per Footprinting Reaction
Binding Buffer	25 $\mu$ l
Labeled probe (15fmol/ $\mu$ l in TE buffer)*	5 $\mu$ l
Nuclease-Free Water	8 $\mu$ l

\*Use a singly end-labeled probe with a specific activity of 15,000–20,000cpm per 1–20pmol.

- Combine 38 $\mu$ l of footprinting assay reagent mix and 12 $\mu$ l of rhSP1 (diluted in SP1 Dilution Buffer). Use 1 $\mu$ g of SP1 per footprinting reaction to produce a strong footprint (see Figure 1).
- Mix by pipetting and incubate on ice for 10 minutes.
- Add 50 $\mu$ l of room temperature 5mM CaCl<sub>2</sub>/10mM MgCl<sub>2</sub> solution and incubate for 1 minute at room temperature.
- Add 3 $\mu$ l of diluted RQ1 RNase-Free DNase (5 $\mu$ l RQ1 RNase-Free DNase diluted into 100 $\mu$ l of cold 10mM Tris HCl [pH 8.0]; keep on ice until use) and incubate for 1 minute at room temperature.
- Terminate the reaction by adding 90 $\mu$ l of Stop Solution, which has been warmed to 37°C. Mix well. Extract each reaction with 200 $\mu$ l of phenol/chloroform/isoamyl alcohol (25:24:1), which has been equilibrated with TE buffer and 0.5M NaCl, to inactivate the DNase.
- Transfer 170 $\mu$ l of the upper, aqueous phase to a fresh tube, add 500 $\mu$ l of 100% ethanol, and precipitate for 20 minutes on ice. Centrifuge at 14,000  $\times$  g in a microcentrifuge for 10 minutes.
- Carefully remove the supernatant, wash with 70% ethanol and dry under vacuum.
- Suspend the pellet in 10 $\mu$ l of Loading Solution by vortexing and flicking the tube. Heat at 95°C for 2 minutes and quick chill on ice for at least 2 minutes.
- Load 3 $\mu$ l of the reaction onto a prewarmed 6% denaturing polyacrylamide sequencing gel. Run the gel at 1,500V, 40 Watts in 1X TBE buffer with 1M urea. Expose the gel on film at –70°C with an intensifying screen. Alternatively, process the gel using a phosphorimaging instrument.



**Figure 1. rhSP1 footprint of the SV40 early promoter fragment:** Lane 1,  $\phi$ X174 DNA *Hinf* I Markers; lane 2, no rhSP1 protein; lane 3, 1000ng purified rhSP1.

## III. Composition of Buffers and Solutions

### Binding Buffer (used in footprinting assays)

50mM	Tris-HCl (pH 8.0)
100mM	KCl
12.5mM	MgCl <sub>2</sub>
1mM	EDTA (pH 8.0)
20%	glycerol
1mM	DTT

### Loading Solution (used in footprinting assays)

33mM	NaOH
67%	formamide
1.2M	urea
0.1%	xylene cyanol
0.1%	bromophenol blue

### Gel Loading Buffer (used in gel shift assays)

250mM	Tris-HCl (pH 7.5)
0.2%	bromophenol blue
40%	glycerol

### Gel Shift Binding 5X Buffer (used in gel shift assays)

20%	glycerol
5mM	MgCl <sub>2</sub>
2.5mM	EDTA
2.5mM	DTT
250mM	NaCl
50mM	Tris-HCl (pH 7.5)
0.25mg/ml	poly (dl-dC)•poly(dl-dC)

### SP1 Dilution Buffer

12mM	HEPES (pH 7.5)
50mM	KCl
6mM	MgCl <sub>2</sub>
5 $\mu$ M	ZnSO <sub>4</sub>
2mM	DTT
0.1%	Nonidet® P-40
50%	glycerol

### Stop Solution

200mM	NaCl
30mM	EDTA
1%	SDS
100 $\mu$ g/ml	yeast tRNA

### TE buffer

10mM	Tris-HCl (pH 8.0)
1mM	EDTA