

HeLa cell cytosol extract S100 human

Cat. No.	Amount
PR-780	200 μ l

For *in vitro* use only
Quality guaranteed for 12 months
Store at -80°C

Avoid freeze / thaw cycles

Form

Liquid. Supplied in 20 mM Tris-HCl, pH 7.9, 100 mM KCl, 0.2 mM EDTA, 1 mM DTT, 20% glycerol.

Activity

1-5 μ l is sufficient for a gel mobility shift assay in a 20 μ l reaction, 5-10 μ l is sufficient for *in vitro* splicing assay and 20-50 μ l is sufficient for a protein-protein interaction assay.

Application

The S100 cytoplasmic extract is specifically recommended for

- 1) *in vitro* splicing,
- 2) protein-DNA/RNA and protein-protein interactions
- 3) source of individual splicing factors and other regulatory proteins.

Purity

> 95% by SDS-PAGE.

Description

The development of cell-free systems that accurately process pre-mRNA by splicing, polyadenylation and editing has led to understanding of insights into the mechanisms and the biochemical characteristics of these reactions. Splicing of pre-mRNA requires the presence of specific sequence elements (cis factors) as well as many protein factors. Although the splicing reaction proceeds in the nucleus, many splicing factors have been found existing in cell cytoplasm. Most essential splicing factors including snRNPs, hnRNPs and debranching enzymes are indeed present in HeLa cell cytoplasmic extract and sufficient to support premRNA splicing *in vitro* when complemented with either naturally purified or recombinant essential splicing factor SF2/ASF. In addition, some transcription factors for RNA Polymerase II, such as TFIIF, are also present in HeLa cytosol.

Selected References:

- Padgett *et al.* (1983) Splicing of adenovirus RNA in a cell-free transcription system. *Proc. Natl. Acad. Sci. USA* **80**:5230.
- Krainer *et al.* (1984) Normal and mutant human beta-globin premRNAs are faithfully and efficiently spliced *in vitro*. *Cell* **36**:993.
- Kramer *et al.* (1985) Purification of a protein required for the splicing of pre-mRNA and its separation from the lariat debranching enzyme. *EMBO J.* **4**:3571.
- Krainer A.R. and Maniatis T. (1985) Multiple factors including the small nuclear ribonucleoproteins U1 and U2 are necessary for premRNA splicing *in vitro*. *Cell* **42**:725.
- Krainer *et al.* (1990) Purification and characterization of pre-mRNA splicing factor SF2 from HeLa cells. *Genes Dev.* **4**:1158.
- Ge *et al.* (1991) Primary structure of the human splicing factor ASF reveals similarities with Drosophila regulators. *Cell* **66**:373.
- Mayeda A. and Krainer A.R. (1999) Preparation of HeLa cell nuclear and cytosolic S100 extracts for *in vitro* splicing. *Methods Mol. Biol.* **118**:309.