

HeLa cell nuclear extract for *in vitro* pre-mRNA splicing human

Cat. No.	Amount
PR-778	200 μ g

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

Avoid freeze / thaw cycles

Form

Liquid. Supplied in 20 mM HEPES-Na (pH 7.9), 42 mM $(\text{NH}_4)_2\text{SO}_4$, 0.2 mM EDTA, 0.5 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* transcription assay and 20-50 μ l is sufficient for a protein-protein interaction assay.

Application

The HeLa cell nuclear extract is specifically recommended for 1) *in vitro* splicing, 2) protein-DNA/RNA and protein-protein interactions, and 3) source of individual splicing factors.

Purity

> 95% by SDS-PAGE.

Description

The HeLa cell nuclear extract was prepared as described by Manley et al. and Krainer et al.. Although this extract contains all basal transcription factors, as well as most gene-specific activators and cofactors, it is prepared specifically for the purpose of pre-mRNA splicing and polyadenylation. Therefore, it has been used as the source of individual polyadenylation factors, splicing factors, and for the cell free system to study the mechanism of pre-mRNA processing.

The HeLa cell nuclear extract is specifically recommended for 1) *in vitro* splicing, 2) protein-DNA/RNA and protein-protein interactions and 3) source of individual splicing factors. Although this extract also contains most transcription factors, it is, however, not recommended for *in vitro* transcription assays.

Please use our specific HeLa nuclear extract for the purpose of *in vitro* transcription.

Selected References:

- Krainer *et al.* (1984) Normal and mutant human beta-globin premRNAs are faithfully and efficiently spliced *in vitro*. *Cell* **36**:993.
Kramer *et al.* (1987) Separation of multiple components of HeLa cell nuclear extracts required for pre-messenger RNA splicing. *J. Biol. Chem.* **262**:17630.
Takagaki *et al.* (1989) Four factors are required for 3'-end cleavage of pre-mRNAs. *Genes Dev.* **3**:1711.
Gerke *et al.* (1986) A protein associated with small nuclear ribonucleoprotein particles recognizes the 3' splice site of pre-messenger RNA. *Cell* **47**:973.
Ruskin *et al.* (1984) Excision of an intact intron as a novel lariat structure during pre-mRNA splicing *in vitro*. *Cell* **38**:317.
Rossi *et al.* (1996) Specific phosphorylation of SR proteins by mammalian DNA topoisomerase I. *Nature* **381**:80.
Krainer *et al.* (1990) The essential pre-mRNA splicing factor SF2 influences 5' splice site selection by activating proximal sites. *Cell* **62**:35.