



## Product Information

### Import Ligand, Fluorescent

Sulforhodamine B Labeled Albumin-NLS Peptide

Product Number **I 9906**

#### Product Description

Proteins due to enter the cell nucleus are "labeled" by nuclear localization signals (NLS). The canonical NLS consists of either a single or bipartite stretch of primarily basic amino acids. The NLS is recognized in the cytoplasm by its receptor importin  $\alpha$ . Importin  $\alpha$  forms a complex with another protein, importin  $\beta$ . This interaction then targets the import complex to the nuclear pore complex (NPC). Passage of the import substrate from the NPC to the nuclear interior requires two additional factors: p10 and the small GTPase Ran.<sup>1-4</sup> In the nucleus, the binding of Ran-GTP to importin  $\beta$  releases the transported protein from the complex.<sup>5,6</sup>

A commonly used NLS for semi-synthetic import ligands is the SV-40 large T-antigen NLS, a 13 amino acid peptide comprised of the following sequence CGGGPKKKRKVED. This peptide is covalently linked to rhodamine-labeled albumin enabling observation of the nuclear import process in permeabilized cells by fluorescence microscopy using a filter suitable for TRITC/Cy3.<sup>7-9</sup>

#### Reagents

The product is a clear red/purple solution is supplied 0.2  $\mu$ m filtered in 0.01 M phosphate buffered saline, pH 7.4.

Dye content: 2 to 4 mole rhodamine B per mole bovine serum albumin.

NLS peptide : 8 to 20 mole NLS per mole bovine serum albumin (migrates as a broad band of MW approximately 78 to 100 kDa on SDS PAGE)

#### Storage/Stability

Store at  $-20^{\circ}\text{C}$ .

Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

#### Product Profile

Optimal import into nuclei of digitonin-permeabilized HeLa cells was observed at a concentration of 120  $\mu\text{g/ml}$ .

$\lambda_{\text{ex}}$  560 nm;  $\lambda_{\text{emm}}$  580 nm

#### References

1. Moore, M.S., and Blobel, G., Proc. Nat. Acad. Sci. USA, **91**, 10212-10216 (1994).
2. Paschal, B.M., and Gerace, L., J. Cell Biol., **129**, 925-937 (1995)
3. Moore, M.S., and Blobel, G., Nature, **365**, 661-663 (1993).
4. Melchior, F., et al., J. Cell Biol., **123**, 1649-1659 (1993).
5. Conti, E., and Izaurralde, E., Curr. Opin. Cell Biol., **13**, 310-319 (2001).
6. Gorlich, D., and Kutay, U., Ann. Rev. Cell Dev. Biol., **15**, 607-660 (1999).
7. Melchior, F., et al., Methods Enzymol., **257**, 279-291 (1995).
8. Radu, A., et al., Proc. Natl. Acad. Sci. USA, **92**, 1769-1773 (1995).
9. Schwoebel, E.D., J. Biol. Chem., **273**, 35170-35175 (1998).

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