

## Nucleo-Selection

### For internalisation of nucleotides and nucleic acids into live cells

Cat.-No.	Amount
CPP-S02	each compound allows up to 75 transductions

#### Components

JBS-Nucleoducin	CPP-C02S	75 transductions
Penetratin	CPP-P01S	0.5 mg
HIV-Tat	CPP-P02S	0.5 mg
MPG	CPP-P03S	0.5 mg
MPG	CPP-P04S	0.5 mg
CAD-2	CPP-P05S	0.5 mg
CPPP-2	CPP-P06S	1.2 mg

For **additionally needed components** read the **general manual (2.2.1)**. Take into account equipment and assays to detect and observe internalized cargo e.g. by selective staining, fluorescence microscopy or cell lyses followed by PAGE-electrophoresis and/or blotting.

#### Storage

Dissolve JBS-Nucleoducin and the other CPPs according to the corresponding data sheets and the **general manual (2.2.3)** in sterile and oxygen free water. Use the solution immediately or store aliquotes at -20°C. Please note that the cocktail has proteolytic activity and can form disulfides and S-oxide (Met) when stored in solution. Avoid frequent thawing and freezing of the stock solutions.

#### Application

The set allows to select the most effective and favourable compound for internalization of various nucleotides or nucleic acids into different live cells. Additionally the set enables you to estimate optimum conditions for this cellular uptake.

#### Instructions

The procedures and their background are described in more detail in the **general manual**.

- Cultivate cells
- Wash cultivated cells thoroughly according to the **general manual**

- Adjust amount of stock solution according to the **general manual (2.2.3)**. 3 to 5 µl of stock solution is able to form a non-covalent complex with 1 µg of an unlabelled nucleotide with a MW of 500 Da and 4 positive charges representing a molar ratio of 1:10; for lower/higher negative charges proportionally decrease/increase amount of CPP, respectively,
- For plasmid DNA: Use 2.5 µl of JBS-Nucleoducin stock solution per µg of plasmid. When using single CPPs use a ten-fold excess of positive charges from CPP (see data sheet) over negative charges from DNA. Consider plasmid DNA as a polymeric base pair.
- Calculate needed volumes of buffers, serum-free medium and serum with FCS according to the desired volume
- Perform complex formation and transduction accordingly to the **general manual (2.2.3, 2.2.4)**
- Add DMSO (10%), BSA (0.5 to 1%) and protease inhibitor o-phenanthroline (1 mM, MW = 198.2 Da) and/or aprotinin (50 µg/ml) to the serum-free medium to increase rate and efficiency of transduction
- If necessary add chloroquine (150 µM, MW = 515 Da) to the serum-containing medium to release internalized cargo from vesicles
- Prolong serum-free transduction time from 1 to 6 h if necessary
- After finishing transduction wash the cells thoroughly, use acidic glycine buffer and if microscopically indicated remove cargo from outside of the cell membrane.
- Check the cells either with a microscope (fluorescence) or by FACS-analysis or by functional tests.

#### Selected References

- Handbook of Cell-Penetrating Peptides*, Second Edition, Ed. by Ü. Langel, CRC Taylor and Francis, Boca Raton, London, New York (2007).
- Morris *et al.* (2008) Cell penetrating peptides: from molecular mechanisms to therapeutics. *Bio.Cell* **100**:201.
- Covic *et al.* (2002) Activation and inhibition of G protein-coupled receptors by cell-penetrating membrane-tethered peptides. *Proc Natl. Acad. Sci.* **99**:643.
- Gros *et al.* (2006) A non-covalent peptide-based strategy for protein and peptide nucleic acid transduction. *Biochim. Biophys. Acta* **1758**:384.
- Snyder *et al.* (2005) Recent advances in the use of protein transduction domains for the delivery of peptides, proteins and nucleic acids in vivo. *Expert Opin. Drug Deliv.* **2**:43.
- Deshayes *et al.* (2008) Delivery of proteins and nucleic acids using a non-covalent peptide-based strategy. *Adv. Drug Deliv. Rev.* **60**:537.