Manual

IFTA AG
Certified QMS according to
DIN EN ISO 9001:2000-12
Reg. No. IC 03214 034

Jena Bioscience

JBS Protein Transduction Kit
Cell Penetration

Cat.-No.	Amount
CPP-K01	25 to 200 transductions

Kit Components

JBS-Proteoducin
Cocktail of Cell Penetrating Peptides and proteins for internalization of peptides and proteins (CPP-C01S)

Fluorescent cargo
Atto488-BSA (100 µg, MW = 68 kDa)
Sufficient for up to 200 transduction experiments (CPP-A08)

Auxiliary Compounds
DMSO (25 ml) (CPP-A01S)
Bovine serum albumin (BSA, 1 g) (CPP-A02)
Chloroquine diphosphate salt (1 g); **Harmful!** (CPP-A03)
Aprotinin (5 mg) (CPP-A05S)
o-Phenanthroline (50 mg); **Toxic!** (CPP-A06S)

Wash Buffer
Concentrated acidic glycine buffer (10x, pH 3, 25 ml, dilute 1:10 with sterile water before use) (CPP-A07S)

For **additionally needed apparates and materials** please read **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 the whole content of the vial JBS-Proteoducin (CPP-C01S) in 250 µl sterile and oxygen free water according to the **general manual**. Use the solution immediately or store aliquots at -20°C. Please note that the cocktail has proteolytic activity and can form disulfides and S-oxide (Met) when stored in solution.
Prepare and aliquot stock solutions from the fluorescent cargo Atto-BSA (CPP-A08) 100 µg in 100 µl sterile water and keep aliquots at -20°C.

Avoid frequent thawing and freezing of the stock solutions. Store the other compounds at 4°C. If stored correctly, Jena Bioscience guarantees a shelf life of 6 months.

Application
The Kit allows internalization of peptides/proteins and estimation of optimum conditions for cellular uptake of the fluorescence labelled cargo, Atto488-BSA, into the cell line of your interest. Some components of the transduction cocktail contain a nuclear localization sequence and are therefore able to transport a cargo into the nucleus.
The Kit further contains compounds for increasing rate and efficiency of transduction. DMSO enhances the permeability of cell membranes. BSA protects to some degree the peptide components of the cocktail against enzymatic degradation and stimulates simultaneously their uptake. Cells with secreted or membrane bound high proteolytic activity require the use of the protease inhibitors aprotinin and/or o-phenanthroline. Chloroquine triggers the release of cargo from intracellular vesicles. The complex which is only bound to the outer site of the cell membrane can be removed by repeated washing with acidic glycine buffer, either pure or with heparin.

Instructions
The procedures and their backgrounds are more detailed described in the **general manual**.
- Cultivate your cells
- Wash accordingly to the **general manual**.
- Adjust amount of stock solution accordingly to the **general manual (2.2.3)**. 1 µl of stock solution is able to form a non-covalent complex with 1 µg of a protein with a MW of 100 kDa, 1.5 µl are necessary for complexation of 1 µl Atto-BSA stock solution.
- Calculate needed volumes of buffers, serum-free medium and serum with FCS according to the final volume

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- 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 microscopically indicated 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 cell membrane with heparin (0.1%)
- Check the cells either with a fluorescence microscope or by FACS-analysis or by SDS-PAGE-electrophoresis

Selected References

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