Product Number(s): CP51101

NEUROMICS

Activated Penetratin 1™

Cell Penetrating Peptide for delivery across cell membranes

Application Manual

Offered in Partnership with

MP

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Related Products

Neurons

Name	Catalog #	Type	Size
Cerebellum	PC35103	P8 Primary Rat	1 Cerebellum
Cortex	PC35102	E18 Primary Rat	1 Cortical pair
Hippocampus	PC35101	E18 Primary Rat	1 Hippocampal pair
Hypothalamus	PC35104	E18 Primary Rat	Hypothalamus pair
Mid-brain	PC35107	E18 Primary Rat	1 Mid-brain
Spinal Cord	PC35106	E18 Primary Rat	1 Spinal cord
Striatum	PC35105	E18 Primary Rat	1 Striata pair

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Activated Penetratin 1 Peptide **Molecular Weight:** 2,715.3

Quality Control: Activated Penetratin 1 is delivered HPLC purified. Quality control includes HPLC, capillary electrophoresis and mass spectrometry.

HPLC QC:
Column: Nucleosil C 18 5μ 100A
2.1 mm x 250 mm
Flow Rate: 0.3 ml/min
CH_3CN gradient from 0 to 60% within 60 min.
Detection: 200nm

Capillary Electrophoresis QC:
Citrate buffer: 20 mM, pH 2.5
Voltage: 30 kV
Detection: 200nm

Coupling Penetratin 1 to oligonucleotides

Protocol for coupling to siRNA and antisense oligonucleotides

Oligonucleotides need to contain a thiol function at one end. The thiol function will be involved in the coupling reaction. The Activated Penetratin 1 peptide features an pyridyl disulfide function at its N-terminal end. Equimolar amounts of Penetratin 1 and oligonulceotide are first incubated for 15 minutes at 65°C to eliminate eventual secondary structures and to prevent precipitation. The coupling is then carried out by incubating the mixture for at least one hour at 37°C. Incubation can be carried out in water. After incubation, both molecules are linked together by means of a disulfide bond. Solutions of coupled Penetratin1-oligonucleotide should be aliquoted and kept at -80°C. Use of a reducing medium (e.g. dithiothreitol (DTT)) would cleave the disulfide bond.

Protocol for Coupling to Oligonucleotides

The following protocol has been used for coupling a 20-mer HPLC purified [ß]-oligodeoxynucleotide, having its 5'-end derivatized by a thiol function (-SH) and lyophilized with DTT.

WARNING: Use only degassed solutions

1. Resuspend 10 OD oligonucleotide (4.25 x 10^{-8} moles) of the oligonucleotide in 250 µl of a 0.1 M DTT aqueous solution.
2. Mix by vortexing gently and incubate overnight at 37°C.
3. Add 500 µL degassed water to the Activated Penetratin 1 to prepare a 1 mg/ml solution.
4. Mix by vortexing gently.
5. Remove DTT from the oligonucleotide solution by gel filtration using degassed water as an eluant.
6. Collect the oligonucleotide in 115 µL of the Activated Penetratin 1 solution (4.25 x 10^{-8} moles).

   If precipitation is observed, add 1 or 2 mL methanol (saturated with N_2) and mix by vortexing.

7. Heat at 65°C for 15 minutes in a tightly sealed tube then incubate at 37°C for one hour.
8. If required, evaporate methanol using a centrifugal evaporator.

Monitoring the Coupling Reaction

Coupling is monitored by means of SDS-PAGE

1. Prepare a SDS-PAGE as follows:

   Acrylamide: bis-acrylamide 37.5:1
   Stacking gel: 6% acrylamide solution, 0.1% SDS, Tris 125 mM, pH 6.8
   Lower gel: 15% acrylamide solution, 0.1% SDS, Tris 375 mM, pH 8.8
   Running buffer: Tris 25 mM, 0.1% SDS,Glycine 200 mM, pH 8.5

2. Remove a small aliquot of coupled Penetratin 1-oligonucleotide and add DTT to solution up to a final concentration of o.o5 M.
3. Incubate at 37°C for 1 hour. Load the following samples on parallel lanes (load approximately 10 to 20 µg equivalent Penetratin 1 in each lane):

   Lane 1: Coupled Penetratin 1-oligonucleotide.
   Lane 2: Uncoupled Activated Penetratin 1.
   Lane 3: Sample of reduced Penetratin 1-oligonucleotide in DTT solution

4. Fix the gel using a methanol:water:acetic acid mixture (5:5:1 in volume). Immerse the gel for 30 minutes at room temperature. Staining is achieved using Coomassie Blue G250 at 0.1% in the fixation solution for 45 minutes.
5. Rinse using an acetic acid:ethanol:water solution (1:1:8 in volume) in order to remove excess dye.

The coupled products (lane 1) migrate at a significantly lower rate than the Activated Penetratin 1 (lane 2). Penetratin 1 produced by DTT reduction (lane 3) migrates at the same position as the original activated Penetratin 1. Note that the pyridyl group of the Activated Penetratin 1 can be partially released during electrophoresis, therefore there can be two bands in lane 2 instead of one.

Coupling Penetratin 1 to Peptides

The peptide which has to be tethered to the Penetratin 1 should feature a thiol function. Make sure this function has not been changed into a disulfide bond during storage of the peptide. In this case, the peptide can be reduced to thiol prior to coupling. Reduction can be made for example by resuspending the peptide in a degassed aqueous solution of TCEP [Tris (2-Carboxy Ethyl) Phosphate]. For more details, see reference: Derossi *et al.* 1996. Add an equimolar amount of the Activated Penetratin 1 (which features an S-S Pyridyl function) to the solution and let incubate for approximately 2 hours at room temperature. The conjugated peptides can be subsequently purified by means of HPLC.

Coupling can also be monitored by SDS-PAGE using appropriate Polyacryamide percaentage. For example, a SDS-PAGE with 18% acrylamide can be prepared. Staining can be carried out in the same way as for the analysis of Penetratin 1-oligonucleotide conjugates.