Monolith Protocol MO-P-077

Amyloid-Beta – Protein X

Amyloid-β (Aβ) is a protein involved in Alzheimer's disease (AD) as the main component of the amyloid plaques found in the brains of people with AD. Aβ molecules can aggregate to form flexible soluble oligomers which may exist in several forms. Certain misfolded oligomers (known as 'seeds') can induce other Aβ molecules to also take the misfolded oligomeric form. The oligomers are toxic to nerve cells. It is unresolved how Aβ accumulates in the central nervous system and subsequently initiates the disease of cells. Finding interaction partners of e.g. Aβ is an important step in trying to find specific biological markers of disease status or disease progression. Aβ 1-42, 42-residue fragment of amyloid precursor protein, has been found to be a major constituent of the senile plaques formed in the brains of patients with Alzheimer's disease and late Down's syndrome. Aβ 1-42 readily forms neurotoxic oligomers at physiological pH. The sequence of this peptide corresponds to the sequence of human, bovine, canine, feline, ovine, guinea pig, and rabbit Aβ42.

protein – protein interaction | amyloid-beta | neurodegenerative diseases

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A1. Target/Fluorescent Molecule

Amyloid β-Protein (1-42) (Aβ42)

A2. Molecule Class/Organism

Homo sapiens (Human)

A3. Sequence/Formula

DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA

A4. Purification Strategy/Source

Synthetic peptide
Bachem
4014447

A5. Stock Concentration/Stock Buffer

1 mg/mL | 222 µM
Powder dissolved in hexafluorisopropanol (HFIP)

A6. Molecular Weight/Extinction Coefficient

4.5 kDa

A7. Dilution Buffer

50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS (MO-L001, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* Dye RED-NHS (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Mix 4.5 µL of the 222 µM amyloid- β stock solution with 95.5 µL of Labeling Buffer NHS to obtain 100 µL of a 10 µM amyloid- β solution.
2. Add 30 µL of DMSO to Dye RED-NHS (10 µg) to obtain a ~470 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 6.4 µL of the 470 µM dye solution with 93.4 µL of Labeling Buffer NHS to obtain 100 µL of a 30 µM dye solution (3x protein concentration).
4. Mix amyloid- β and dye in a 1:1 volume ratio (200 µL final volume, 3.2% final DMSO concentration).
5. Incubate for 30 minutes at room temperature in the dark.
6. In the meantime, remove the top cap of the B-Column and pour off the storage solution. Remove the bottom cap and place with adapter in a 15 mL tube.
7. Fill the column with dilution buffer and allow it to enter the packed resin bed completely by gravity flow. Discard the flow through collected. Repeat this step 3 more times.
8. Add 200 µL of the labeling reaction from step 4 to the center of the column and let sample enter the resin bed completely.
9. Add 300 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 600 µL of dilution buffer and collect the eluate.
11. Keep the labeled amyloid- β (~1.7 µM) on ice in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Protein X[1]

B2. Molecule Class/Organism

Homo sapiens (Human)

B3. Sequence/Formula

N/A

B4. Purification Strategy/Source

N/A

B5. Stock Concentration/Stock Buffer

145 µM

B6. Molecular Weight/Extinction Coefficient

33 kDa

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 145 µM protein X solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
2. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
3. Mix 2 µL of labeled amyloid- β (~1.7 µM) with 198 µL of dilution buffer to obtain 200 µL of ~16 nM amyloid- β.
4. Add 10 µL of this solution to each tube from **16** to **1** and mix by pipetting.
5. Load capillaries directly.

[1] As the data in this protocol hasn't been published yet, the target is still confidential and called 'protein X'. As soon as the data is published, the real target name will be added.

D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

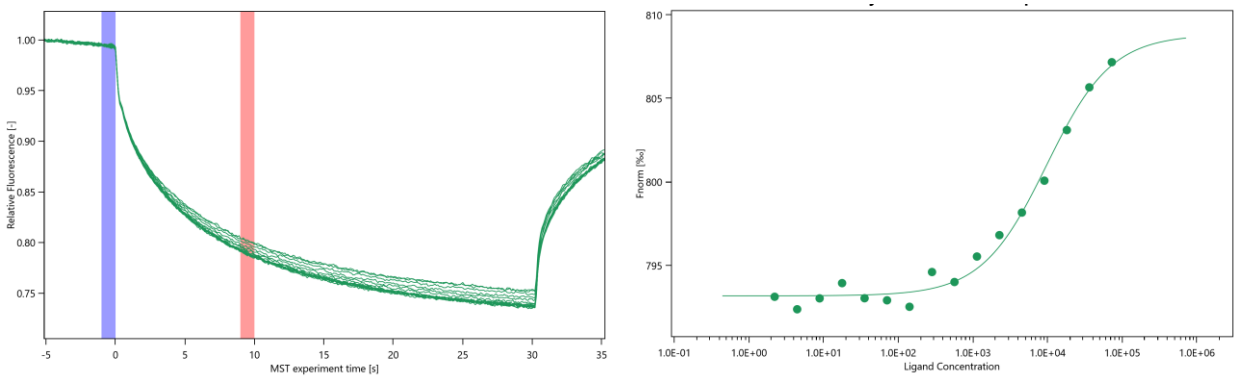
NT.Control | MO.AffinityAnalysis (NanoTemper Technologies GmbH)

D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20
8 nM amyloid- β | 72.5 µM – 2.2 nM protein X | 22°C | medium MST power | 70% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

K_d = 9 µM



D5. Reference Results/Supporting Results

N/A

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