



## POTENTIATING THE ITALIAN CAPACITY FOR STRUCTURAL BIOLOGY SERVICES IN INSTRUCT-ERIC

### PROTOCOL FOR EXPRESSION AND PURIFICATION OF Ab40-42

*IO2.1.4 Expression protocols*

*Activity 2.1: Development of a platform for a high-throughput protein expression*

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*Period: B9 (March-April 2024)*

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#### **Call for proposals MUR n. 3264 in date 28/12/2021**

**Mission 4** – Education and Research Component 2: from research to business

**Investment 3.1** Fund for the realisation of an integrated system of research and innovation infrastructures

**Action 3.1.1** Creation of new research infrastructures, strengthening of existing ones and their networking for Scientific Excellence under Horizon Europe

RI : INSTRUCT-ERIC – Integrated Structural Biology Infrastructure

ESFRI domain: Health and Food

RI at high priority

Funding Admission DD MUR n. 115 in date 21/06/2022

Starting Date: 1 November 2022

Duration of the project: 30 month

ID Proposal: IR0000009

CUP: B53C22001790006

## Protocol for Expression and purification of A $\beta$ 40-42

### Buffers:

Lysis buffer: 10 mM Tris, 1 mM EDTA, protease inhibitor tablet pH 8.0

Solubilization buffer (or Buffer A AIEX): 10 mM Tris, 1 mM EDTA, 8 M Urea, pH 8.0

AIEX Buffer B: 10 mM Tris, 1 mM EDTA, 8 M urea, 1 M NaCl, pH 8.0

SEC buffer: 50 mM (NH<sub>4</sub>)OAC pH 8.5

Single <sup>15</sup>N labelling is done with M9 supplemented with 1/1.2 g of labelled (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> x L.

### Expression of A $\beta$ 40-42

Transformed BL21(DE3)pLyss *E. Coli* cells were grown overnight in 100/200 ml of LB, with antibiotics, at 37°C. Before doing the inoculation, OD<sub>600</sub> was checked and a 1:20 dilution has been done. The inocule was grown at 37/39 °C until OD<sub>600</sub> 0.6 and the protein was overexpressed for 5 h at 39°C before being spun down at 5000 rpm for 20 mins at 4°C.

The cell pellets were dissolved into lysis buffer (50 ml per 1 L of cell culture), sonicated, in ice, for 5 min at 60% amplitude (5 sec on, 20 sec off) and spun down at 35000 rpm for 20 mins at 4°C. Then, the supernatant was discarded and the pellet is saved and dissolved, again, into lysis buffer in order to make another centrifugation at 35000 rpm for 20 mins at 4°C. Again, the pellet was saved and dissolved into solubilization buffer and leave, under stirring, at 4°C O/N. The day after, the protein solution was spun down at 35000 rpm for 20 mins at 4°C and the supernatant was loaded onto the HiPrep QFF 16/10 column and the protein was eluted with a mix of buffer A/B. The fractions of main peaks were collected and were undergone SDS-PAGE. Then, the interested AIEX fractions were joined together and an amount of GndHCl was added in order to reach final conc of 6 M GndHCl. In meantime, the Superdex 30 pg 16/600 (or Superdex 75 pg 16/600) was washed with milliQ H<sub>2</sub>O and equilibrated O/N with 50 mM NH<sub>4</sub>OAC pH 8.5 solution to be ready for running the chromatography the day after. 10 uL of each single fraction were taken for SDS-PAGE gel and, then, were merged and then readily flash-frozen with low temperature nitrogen and lyophilized (if necessary). The concentration of the protein was checked with UV-Vis spectrophotometer.

ξ<sub>280</sub> Epsilon at 280 nm: 2073

ξ<sub>260</sub> Epsilon at 260 nm: 3198

The protein elute around 20% of buffer B during AIEX

Volume elution of A $\beta$ 40\_42 with Superdex 30 pg 16/60: around 70-75 ml

Volume elution of A $\beta$ 40\_42 with Superdex 75 pg 16/60: around 82-85 ml