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IF Labeling Protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details the Labelling of Immunofluorescent Protein.

Materials

- Alexa Fluor™ 488 Microscale Protein Labeling Kit **Thermo Fisher Catalog #**A30006

Immunofluorescent Protein Labeling Protocol

17m 15s

1 Alex Fluor 488 Microscale Protein Labelling Kit (Invitrogen, A30006).

1.1 According to manufacturer protocol, AF 488 reactive dye has Tetrafluorophenyl (TFP) ester moiety that is more stable in solution than the commonly used succinimidyl (NHS) ester. TFP ester react efficiently with primary amines of protein to form a stable dye-protein conjugate and is independent of pH between 4 and 10.

1.2 Single kit can label a total of 200 µg of αS preformed fibrils.

1.3 Dilute Hu-WT-αS fibrils (PFFs) to a concentration of 1 µL in PBS followed by sonication (1 Sec ON/1 Sec OFF) for 00:02:00 with 20% amplitude.

2m

1.4 Mix diluted PFFs with component B (NaHCO3), then added freshly prepared reactive dye (component A) and incubated for 00:15:00 at Room temperature .

15m

1.5 Calculate amount of reactive dye using dye/protein molar ratio of 9.

1.6 Remove excess dye by resin gel (component E) purification.

1.7 Place conjugated reaction mixture of PFFs and reactive dye on the center of the resin bed surface, centrifuge at 16000 x g, 00:00:15 .

15s

1.8 Collect the filtrate as labeled PFFs.