

## Labeling Amyloid Beta with pHrodo Red

### Introduction

Alzheimer's Disease is characterized by irreversible progressive neural degeneration associated with the formation of amyloid plaques primarily composed of aggregated amyloid beta peptides.

Amyloid beta (A $\beta$ ) consists of peptides of variable length, typically 36-43 amino acids, which are formed when amyloid precursor protein is processed by beta and gamma secretases. In particular, variant A $\beta$  1-42 has been shown to be especially prone to self-aggregating, leading to accumulation of an array of low and high molecular weight aggregates. Assaying the consequences of A $\beta$  aggregation may uncover therapeutic agents and methods for understanding and preventing the devastating effects of aberrant A $\beta$  on human health and disease.

This protocol describes the preparation of A $\beta$  aggregates and labeling with pHrodo Red, an amine-reactive and pH-sensitive dye. The labeled aggregates increase in fluorescence as the aggregates are involved in phagocytosis and the pH within the cell becomes more acidic, thus allowing phagocytosis to be quantified using microscopy, live imaging or other fluorescence detection technologies.

### Required Equipment and Consumables

The following equipment and consumables are required in addition to the materials specified in the iCell Microglia Quick Guide.

Item	Vendor(s)	Catalog Numbers
<b>Equipment</b>		
Sonicator Bath (optional)	Multiple Vendors	
Microcentrifuge	Multiple Vendors	
<b>Consumables</b>		
0.2 $\mu$ M PES Filter Unit	Multiple Vendors	
Beta-Amyloid (1-42) Aggregation Kit, 0.25mg <sup>1</sup>	rPeptide	A-1170-02
Dimethyl sulfoxide (DMSO), ACS Reagent	MilliporeSigma	472301
Hank's 1X Balanced Salt Solution without Ca <sup>2+</sup> , Mg <sup>2+</sup> , phenol red (HBSS)	GE Healthcare Life Sciences	SH30588.02
Methanol	Multiple Vendors	
Microcentrifuge Tubes	Multiple Vendors	
pHrodo™ Red, Succinimidyl Ester	ThermoFisher Scientific	P36600
Sodium Bicarbonate, Powder	MilliporeSigma	S5761

<sup>1</sup> Kit contents include: A-1167 Beta Amyloid, 5mM Tris, 10mM NaOH, 10X TBS pH 7.4, HPLC water and 400  $\mu$ M Thioflavin.

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## Methods

The following procedure details the reconstitution and aggregation of A $\beta$  (1-42) and subsequent labeling of aggregates with pHrodo Red.

### Day 1: Preparing A $\beta$ Aggregates

1. Equilibrate the Beta-Amyloid (1-42) Aggregation Kit to room temperature.
2. Add 250  $\mu$ l of 10 mM NaOH to the A $\beta$  vial. Swirl the tube to ensure hydration of the lyophilized material.
3. Transfer the rehydrated A $\beta$  to a sterile 1.5 ml centrifuge tube.
4. Add 250  $\mu$ l of HPLC water to the 1.5 ml centrifuge tube.
5. Add 56  $\mu$ l of 10X TBS pH 7.4 to neutralize the solution. Mix well by pipetting up and down several times.

**Note:** *This yields approximately 560  $\mu$ l of 100  $\mu$ M A $\beta$  solution.*

6. Incubate the prepared A $\beta$  solution at 37°C overnight to allow for aggregation.

**Note:** After incubation, A $\beta$  aggregates appear cloudy.

### Day 2: Labeling A $\beta$ Aggregates with pHrodo Red

1. Prepare 0.1 M sodium bicarbonate by diluting 250 mg sodium bicarbonate in 30 ml of sterile water.
2. Filter the solution through a 0.2  $\mu$ M filter unit.
3. Centrifuge the A $\beta$  solution at 16,000 x g for 1 minute to collect the aggregates.
4. Aspirate the supernatant carefully to avoid disturbing the pellet.
5. Add 1 ml of HBSS to the tube to rinse the aggregates and pipette to mix.
6. Centrifuge the tube at 16,000 x g for 1 minute to collect the aggregates.
7. Aspirate the supernatant carefully to avoid disturbing the pellet.
8. Add 200  $\mu$ l of 0.1 M sodium bicarbonate to the aggregates and resuspend by pipetting several times.
9. Prepare 10 mg/ml pHrodo Red dye by diluting 1 mg of pHrodo Red in 100  $\mu$ l of DMSO. Mix well by vortexing.
10. Add 36  $\mu$ l of the 10 mg/ml pHrodo Red dye to the A $\beta$  aggregate suspension to initiate the labeling reaction. Pipette several times to mix well.

**Note:** *The final volume is ~250  $\mu$ l at a ratio of ~10 dye molecules per A $\beta$  molecule.*

11. Incubate the reaction tube for at least 1 hour at room temperature in the dark.
12. Centrifuge the reaction tube at 16,000 x g for 1 minute
13. Aspirate the supernatant.  
**Note:** *The A $\beta$  pellet will be dark purple and the supernatant will be light reddish-purple.*
14. Add 1 ml of methanol to the reaction tube, to remove excess dye, and vortex for 5 - 10 seconds.

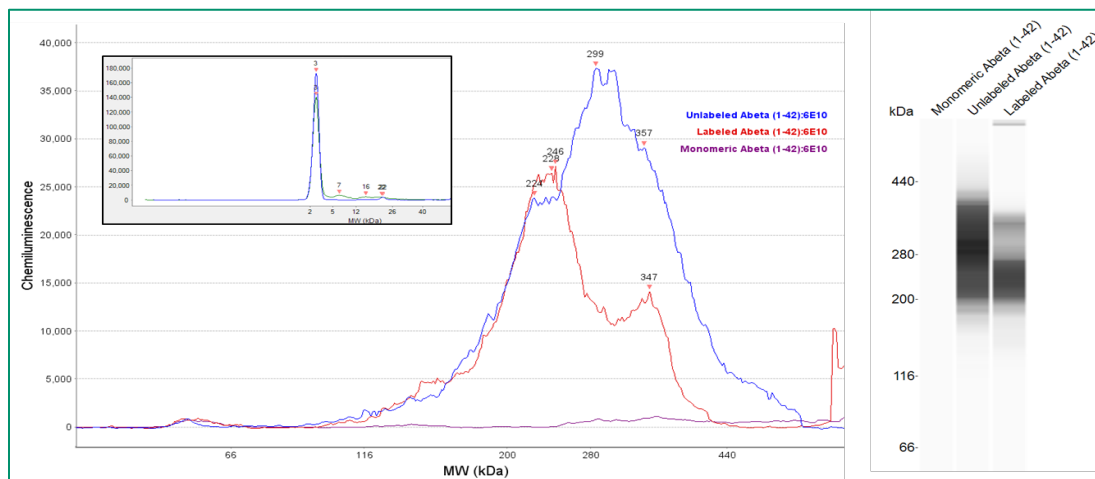
15. Centrifuge the reaction tube at 16,000 x g for 1 minute.
16. Aspirate the supernatant.
17. Add 200  $\mu$ l of HBSS to the reaction tube and pipette to mix.
18. Add an additional 800  $\mu$ l of HBSS to the reaction tube and vortex for 5 – 10 seconds to wash away excess dye.
19. Centrifuge the reaction tube at 16,000 x g for 1 minute.
20. Aspirate the supernatant and wash an additional three times by adding 1 ml of HBSS to the reaction tube, vortexing for 5 – 10 seconds, centrifuging the reaction tube at 16,000 x g for 1 minute and aspirating the supernatant.
21. After final wash, aspirate the supernatant.
22. Add 200  $\mu$ l of HBSS to the reaction tube and pipette to mix.  
*Optional: Place the tube in a sonicator bath at room temperature for 5 – 10 minutes to improve the consistency of the A $\beta$  suspension.*
23. Use pHrodo Red-labeled A $\beta$  immediately or store single use aliquots at -80°C for up to 6 months.

## Using pHrodo Red-labeled A $\beta$ with iCell Microglia

Refer to the Application Protocol Measuring Microglia Phagocytosis: Kinetic Imaging on the IncuCyte accessible at [https://fujifilmcdi.com/assets/CD1\\_iCellMGL\\_IncuCyte\\_AP.pdf](https://fujifilmcdi.com/assets/CD1_iCellMGL_IncuCyte_AP.pdf) to measure phagocytosis of the pHrodo red-labeled A $\beta$  by the iCell Microglia.

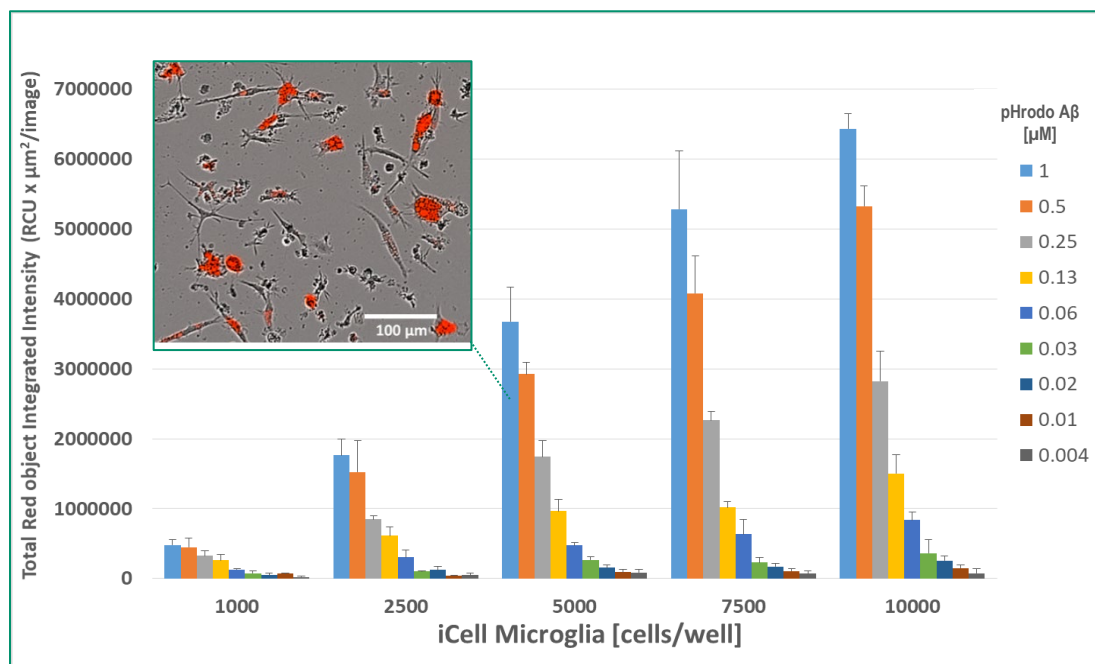
## Example Data

Results displayed in Figures 1 and 2 illustrate representative pHrodo Red-labeled A $\beta$  characterization and phagocytosis of pHrodo Red A $\beta$ .



**Figure 1. A $\beta$  (1-42) Aggregates Protein Characterization**

Aggregates were analyzed by a Simple Western using Wes (ProteinSimple) and anti- $\beta$ -Amyloid Antibody (BioLegend # 803001; Clone 6E10).



**Figure 2: iCell Microglia Phagocytosis of pHrodo Red-Labeled Aβ Aggregates**  
*iCell Microglia phagocytose pHrodo-red labeled aβ aggregates in a titration-dependent manner.*


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**Revision History**

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