

# **PINEWOOD SCIENTIFIC SERVICES INC**

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## **Preparation suggestions for ALEXA 488 labeled SOLUBLE FLAER, cat. No. FL1S.**

The reagent has been shipped AS A LIQUID in 0.5 ml of buffer at a concentration of  $10^{-6}$  M. It can be stored at 4 °C in the refrigerator. IT DOES NOT NEED TO BE FROZEN, BUT as with other fluorescent materials, it should be protected from long exposure to light.

Liquid FLAER is a new reagent and its shelf life has not been firmly established. The reagent is intended for research purpose only.

We suggest using the reagent at a final concentration of  $5 \times 10^{-8}$  M (for example, by diluting 5  $\mu$ L into a final volume of 100  $\mu$ L containing cells), however you might wish to try other concentrations for your own application. We recommend that you do not dilute the reagent before use.

## **Suggested simple method for using SOLUBLE FLAER to detect GPI-anchored protein-deficient cells by flow cytometry. See below for methods used in PNH diagnosis.**

$1 \times 10^6$  cells are washed once in cold phosphate-buffered saline (PBS) by centrifugation at 1100 rpm at 4°C for 10 minutes and resuspended in 250  $\mu$ L of cold PBS. FLAER is added to a final concentration of 10 nmol/L ( $5 \times 10^{-8}$ M). The mixture is incubated in the dark on ice for 20 minutes. The cells are then washed and resuspended in cold PBS, and fixed by adding an equal volume of 2% paraformaldehyde. Analysis of FLAER binding is performed using a flow cytometer equipped with 488-nm argon ion laser. A sample of cells known for GPI-anchor expression is stained at the same time and used as a positive control to discriminate the FLAER negative population in the tests.

**There are a number of published methods describing the detection of PNH using FLAER in combination with labeled antibodies. One example is Sutherland et al. in Cytometry Part B (Clinical Cytometry) 72B: 167-177 (2007). We can provide a detailed protocol upon request.**

Please contact us if you have any questions.

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