

# **PINEWOOD SCIENTIFIC SERVICES INC**

(Formerly Protox Biotech)  
210 Denison Rd.  
Victoria, BC  
V8S 4K3  
Canada

## **Preparation suggestions for Alexa-488 Proaerolysin (FLAER) cat. No. FL2.**

The reagent is intended for research purpose only. It has been shipped as a powder. **Please use care when opening the vial to avoid losses.** We suggest that before use you dissolve the powder in 1 mL of an appropriate buffer such as PBS. This will give a **stock solution** with a concentration of  $10^{-6}$ M. Be sure to rinse the vial cap with the buffer as it may contain some of the reagent. Aliquot the solution in appropriate volumes and store the aliquots frozen and in the dark until use. Repeated freezing and thawing should be avoided. **Alternatively, you may wish to store the stock solution in the refrigerator. In this case you may wish to add a preservative such as sodium azide.**

$5 \times 10^{-8}$  M has proven to be a useful final concentration for flow cytometry (a 1 to 20 dilution of the stock), however you might wish to try other concentrations for your own application.

## **Suggested simple method for using 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  $4^{\circ}$ C for 10 minutes and resuspended in 250  $\mu$ L of cold PBS. FLAER is added to a final concentration of 50 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. 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.

Tom Buckley  
Tel. 250-598-6822, fax. 250-598-6877  
Tbuckley@protoxbiotech.com