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FLAER: Alexa Fluor® 488 proaerolysin

CE

FL1S-C, 25 µg
FL2S-C, 50 µg

For *In Vitro* Diagnostic Use

Intended Use

FLAER is used in a clinical laboratory setting for multi-parameter flow cytometry along with antibodies (including CD45, CD33, CD24, CD15 and CD14) to detect PNH clones (FLAER-negative cells) within the monocyte and granulocyte (cell) lineages.[6,7,9] The result is a sensitive an accurate test that can be used in combination with the CD55/CD59 assay for detecting PHN clones in red blood cells.

Summary:

Paroxysmal nocturnal hemoglobinuria (PNH) is a stem cell disorder caused by a mutation of the gene involved in the synthesis of the GPI (glycosylphosphatidylinositol) anchor of a group of surface proteins on circulating cells. Affected cells are sensitive to complement-mediated hemolysis and this may lead to life-threatening thrombosis, chronic kidney disease, pulmonary hypertension, end organ damage, ischemic bowel disease, hepatic failure, and anemia.

Identifying PNH patients early in the course of their disease may offer the best opportunity for long-term management. In the past, PNH has been challenging to identify effectively. However, in recent years impressive strides have been made in the understanding of PNH pathology, accompanied by greatly improved detection techniques, including multiparametric flow cytometry using FLAER.

About FLAER

FLAER is an Alexa Fluor® 488 labeled variant of aerolysin, a unique protein that binds tightly and specifically to mammalian GPI anchors. FLAER will not bind to PNH cells because they do not produce the anchor. Before FLAER, detection of PNH clones by flow cytometry relied on fluorescently labeled antibodies to GPI-linked proteins such as CD59 and CD55. These antibodies do not bind with high affinity, so that small PNH clones are not detected. Also, they each screen for the absence of a specific protein, rather than loss of the GPI anchor, and therefore there is the risk of false negative results. Since FLAER binds to the GPI anchor itself, only PNH cells, which lack the anchor, will be negative. And since FLAER binds with high affinity, very small PNH populations can be detected.

Continued Overleaf….

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Formulation:

FL1S-C contains 25 µg of FLAER in 0.5 ml PBS-albumin (50 tests), 0.05% Sodium Azide
FL2S-C contains 50 µg of FLAER in 1.0 ml PBS-albumin (100 tests), 0.05% Sodium Azide

The concentration of FLAER in the supplied stock solution is 10^{-6} M. The solution also contains 5 mg/mL albumin, added in order to maximize stability.

Storage and Stability:

The stock solution may be kept in the refrigerator (+2-8°C) protected from light for 2 years from date of manufacture.

Usage:

5×10^{-8} M has proven to be a useful final concentration of FLAER for flow cytometry[4,6,7] (this represents a 1 to 20 dilution of the stock directly into the test sample), however one may wish to try other concentrations to fit the individual assay set up.

***Optimal dilutions of the other antibodies in the test sample must be determined by the investigator.**

There are a number of published methods describing the detection of PNH using FLAER. See references 6, 7, and 9.

Note: FLAER is used in combination with antibodies (including CD45, CD33, CD24, CD15 and CD14) to detect PNH clones (FLAER-negative cells) within the monocyte and granulocyte (cell) lineages. FLAER can be used in a 3, 4, 5 or 6 colour flow cytometry assay, depending on how many antibodies that a given laboratory will use in combination with FLAER. Each lab needs to determine their optimal assay set up.

The appropriate isotype controls should be used in any experiment along with a PNH positive control sample.

Limitations:

1. Each clinical laboratory needs to develop their own assay set up, combination of assays and the cut-off levels in those assays to accurately detect PNH.
2. Regents/antibodies can be used in different combinations; therefore laboratories need to become familiar with the performance characteristics of each reagent/antibody in relation with the combined markers in normal and abnormal samples.
3. Reagent data performance is based on EDTA-treated blood. Reagent performance may be affected by the use of other anticoagulants.

Reagents and materials required but not provided:

1. Flow Cytometer
2. Test tubes for flow cytometer
3. Pipette with disposable tips
4. Vortex mixer
5. Centrifuge
6. PBS (phosphate buffered saline)/ 3% albumin
7. Red blood cell lysis reagent
8. A PNH positive control sample

Continued Overleaf….

Staining Procedure:

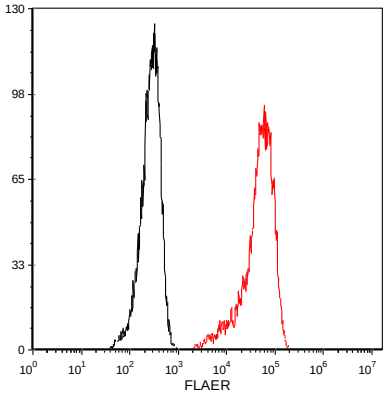
1. Lyse RBCs from 100 µL of anticoagulated (EDTA) peripheral blood with the appropriate lysing buffer, wash, and resuspended in100 µL of phosphate-buffered saline (PBS)/3% albumin.
2. Add appropriate amounts of antibodies and 5 µL of FLAER (stock solution).
3. Vortex and incubate for 15 minutes at room temperature.
4. After washing, then analyze the sample on an appropriate flow cytometer.

Performance:

On healthy individuals, FLAER binds to essentially all human lymphocytes, monocytes and granulocytes. In our testing, lymphocytes were labelled with FLAER as described above.

The histogram below outlines count versus fluorescence intensity of FLAER labeled lymphocytes from a healthy individual (acquisition is performed using the Accuri C6 and analyzed in FCS Express).

FLAER (red histogram) at a dilution of $5.0x10^{-8}$ M vs. unstained cells (black histogram).



Expected Values:

In our testing, lymphocytes from healthy individuals were labelled with FLAER as described above. The results obtained for the count of the positive events of interest with FLAER are as follows:

Lymphocytes	Number	Mean (% Staining)	SD (% Staining)	CV (%)
FLAER +	10	99.92	0.15	0.15

Characterization:

To ensure consistent high-quality reagents, each batch of FLAER is tested for conformance (to ensure that it binds GPI anchors and that there is a minimum shift between positive and negative samples).

Warnings and Precautionary statements:

Product contains Sodium Azide. This Chemical is poisonous and hazardous. Handling should be done by trained staff only.

FLAER is produced by labeling an inactive variant of the protein proaerolysin with Alexafluor® 488 succinimidyl ester, provided under agreement with Life Technologies Corporation.

Continued Overleaf….

References:

1. Buckley JT. (1990) **Purification of cloned proaerolysin released by a low protease mutant of Aeromonas salmonicida**. Biochem Cell Biol. 68(1):221-4.
2. Buckley JT. (1992) **The channel-forming toxin aerolysin**. FEMS Microbiol Immunol. 5(1-3):13-7.
3. Rossjohn J. et al. (1998) **Movement of a loop in domain 3 of aerolysin is required for channel formation**. Biochemistry. 37(2):741-6.
4. Brodsky RA. et al. (2000) **Improved detection and characterization of paroxysmal nocturnal hemoglobinuria using fluorescent aerolysin**. Am J Clin Pathol. 114(3):459-66.
5. Burr SE, Diep DB, Buckley JT. (2001) **Type II secretion by Aeromonas salmonicida: evidence for two periplasmic pools of proaerolysin**. J Bacteriol. 183(20):5956-63.
6. Sutherland DR. et al (2007) **Diagnosing PNH with FLAER and multiparameter flow cytometry**. Cytometry B Clin Cytom. 72(3):167-77.
7. Sutherland DR. et al. (2009) **Use of a FLAER-based WBC assay in the primary screening of PNH clones**. Am J Clin Pathol. 132(4):564-72.
8. Brodsky RA. (2009) **How I treat paroxysmal nocturnal hemoglobinuria**. Blood. 113(26):6522-7.
9. Borowitz MJ. et al; Clinical Cytometry Society. (2010) **Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry**. Cytometry B Clin Cytom. 78(4):211-30.

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