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CERTIFICATE OF ANALYSIS

PRODUCT:	PE-IgG conjugate, goat anti-human IgM, mu-specific
CODE:	GTIM-001
LOT NUMBER:	09269041
AMOUNT:	1 mL
CONCENTRATION:	1 mg/mL
EXPIRATION:	SEP 2006
ORIGIN:	Goat anti-human IgM mu, affinity purified, U.S.A. origin R-phycoerythrin, U.S.A. origin
BUFFER:	10 mM sodium phosphate, 140 mM sodium chloride, pH 7.3, stabilizer 15 mg/mL BSA
PRESERVATIVE:	0.05% (w/v) sodium azide.
STORAGE:	2-8^{o}C
APPEARANCE:	Pass
UV/VIS:	Pass
FLUORESCENCE:	Pass
FRET:	Pass
SHIPPING:	Ice Packs

Alvydas Ozinskas
Manager QC/QA Bioproducts

FLUORESCENT CONJUGATE SOLUTION
PE-IgG, GOAT ANTI-HUMAN IgM, Mu-SPECIFIC
MOSS INC. PRODUCT NO. GTIM-001

INTRODUCTION

Moss Inc. Phycoerythrin-IgG (PE-IgG) conjugates excel in diagnostic, molecular and cellular fluorescence assays based on antibody detection. Moss PE-IgG conjugates are manufactured reproducibly in homogeneous, liquid stable form and are suitable for use on various immunoassay, flow cytometry, and multiplexing platforms such as the Luminex and microarrays. Moss PE-IgG conjugation technology produces conjugates that result in exceptional signal-to-noise ratios, high titers, and the conjugates can also be customized to maximize performance for specific platform applications.

The goat IgG, anti-human IgM, mu-specific is affinity purified and binds the heavy chain or mu-chain of human IgM. R-Phycoerythrin is a pink-colored protein purified from seaweed. The intense R-PE absorption maximum at 566 nm ($E_{566\ nm}$ = 1.96 x 10^6 M^{-1} cm^{-1}) and the strong relative maxima near 545 nm and 498 nm provide multiple opportunities to select excitation wavelengths. The emission maximum is at 578 nm with a high quantum yield. Product No. GTIM-001, available from **Moss Inc.**, is a highly sensitive single component reagent that is ready to use for the quantitative detection of human IgM bound to a solid phase such as a microsphere or microarray, a biological cell, or in free solution. **GTIM-001 is stable for 28 months when refrigerated** and is not appreciably sensitive to normal laboratory light over the course of a typical usage and detection cycle. It should be refrigerated dark when not in use, and exposure to sunlight should be avoided.

USAGE SYNOPSIS

Upon completion of a procedure requiring the detection of human IgM, the appropriate dilution of GTIM-001 is added. Dilutions of PE-anti-hIgM can be made using Moss Inc. PE diluent, Product No. PECD-100, -500, and -1000. If the required dilution is unknown, dilutions of GTIM-001 can be made beginning with a 1:50 dilution. Appropriate fluorescence excitation and emission wavelengths and associated parameters can be selected according to instrument capabilities. Excitation wavelengths selected near the absorption maxima of 566 nm, 545 nm, and 498 nm result in higher fluorescence emission intensities.

REAGENT PROVIDED

GTIM-001 SOLUTION: Contains 1 mg/mL PE-IgG, goat anti-human IgM, mu-specific in buffer containing 10 mM sodium phosphate, 140 mM sodium chloride, pH 7.3. The solution is stabilized with 15 mg/mL BSA and preserved with 0.05% sodium azide.

Store dark at refrigerator temperatures, 2-8°C.

Protect from exposure to sunlight.

AVAILABLE PE-Anti-hIgM DILUENT

PECD-100 SOLUTION: Contains a proprietary phosphate buffered saline solution, pH 7.3 with 0.05% sodium azide preservative.

Store at refrigerator temperatures, 2-8°C.

QC USING FRET METHOD

The Moss Inc. FRET test was developed for internal use to test PE-IgG conjugates, and of course any suitable methodology may be used to test PE-IgG conjugates. PE-anti-hIgM serves as the energy transfer donor in the test. The energy transfer acceptor conjugate is Cy5-human IgM (Cy5-hIgM) and is prepared by Moss Inc. The FRET test is a homogeneous assay, which means that there are no separation or wash steps. The reagents are mixed together in microtiter plate wells, and then the fluorescence is measured after 15 minutes.

FRET TEST PROCEDURE USING 96 WELL BLACK MICROTITER PLATES

The assay is conducted in a black microtiter plate. The plate is divided into an upper half (Rows A-D) and a lower half (Rows E-H). Each column (1-12) has n=2 donor alone replicates (each well in rows A and B has PE-anti-hIgM and buffer, 100 µL final volume). Each column also has n=2 donor plus acceptor replicates (each well in Rows C and D has PE-anti-hIgM and Cy5-hIgM, 100 µL final volume). One or more columns can be used for the buffer alone control (no donor or acceptor) and for the acceptor alone control (no donor) with 100 µL final volume for each well.

1. Prepare 0.01 mg/mL dilutions each of PE-anti-hIgM and Cy5-hIgM using a buffer of 10 mM PBS, pH 7.3, 1.5 mg/mL BSA. It is convenient to use a second microtiter plate to prepare the PE-anti-hIgM dilutions (300 µL each) for use with a multi-channel pipettor.
2. Add 50 µL of buffer to Rows A, B E, and F.
3. For the upper half of the plate, add 50 µL of one set of PE-anti-hIgM donor conjugates to each well in rows A, B, C and D. A different PE-anti-hIgM donor conjugate can be used for each column, and in this case n=2. We usually use the same PE-anti-hIgM conjugate for 4 columns, so n=8 in this case.
4. To the lower half of the plate, add 50 µL of the next set of PE-anti-hIgM donor conjugates to each well in rows E, F, G and H.
5. Add 50 µL of Cy5-hIgM acceptor to each well in Rows C, D, G and H.
6. Mix plate on orbital shaker at 700 RPM for 5-7 seconds.
7. Read plate after 15 minutes at ambient temperature using a fluorescence microtiter plate reader.

Data Processing:

1. Average the 15 minute readings for each PE-anti-hIgM conjugate, separately for the donor alone (Fo), and for the donor plus acceptor (F). The CVs are generally 1-3%.
2. Correct for background by subtracting the fluorescence readings for the buffer alone control from Fo, and by subtracting the fluorescence readings for the buffer plus acceptor control from F.
3. Calculate F/Fo for each PE-anti-hIgM conjugate, using F and Fo values that were corrected for background. Normalize the data if required. Prepare a table, graph, or chart.

Significance of results:

1. No binding results in no energy transfer and F/Fo = 1.00, theoretically.
2. A binding interaction between donor and acceptor results in fluorescence resonance energy transfer and F/Fo < 1.00.
3. The smaller the F/Fo value, the stronger the binding interaction.
4. Variations of the assay are possible by adding binding ligands to generate an analyte titration curve.
5. In general, for a given PE-anti-hIgM conjugate, the range between replicates for F/Fo = 0.03.

The method described is comparable to that used at **Moss Inc.** Variations in test parameters require standardization by the user.