FLUORESCENT CONJUGATE SOLUTION
STREPTAVIDIN-PHYCOERYTHRIN
MOSS INC. PRODUCT NO. SAPE-001

INTRODUCTION

Moss Inc. streptavidin-phycoerythrin conjugates excel in diagnostic, molecular and cellular fluorescence detection assays based on biotin labeling. Moss SAPE 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 SAPE 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.

Streptavidin is a tetrameric protein isolated from the bacterium *Streptomyces avidinii* that exhibits an extremely high binding affinity for biotin ($K_a = 10^{15}\ M^{-1}$) with four biotin binding sites. R-Phycoerythrin is a pink-colored protein purified from seaweed. The intense absorption maximum at 566 nm ($E_{566\ nm} = 1.96 \times 10^6\ M^{-1}\ cm^{-1}$) 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. The high biotin affinity of streptavidin combined with the intense fluorescence signal of phycoerythrin make SAPE conjugates among the most sensitive fluorescence detection reagents. Product No. SAPE-001, available from **Moss Inc.**, is a highly sensitive single component reagent that is ready to use for the quantitative detection of biotin bound to a solid phase such as a microsphere or microarray, a biological cell, or in free solution. **SAPE-001 is stable for 36 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 biotin, the appropriate dilution of SAPE-001 is added. Dilutions of SAPE can be made using Moss Inc. PE diluent, Product No. PECD-100, -500, and -1000. If the required dilution is unknown, dilutions of SAPE-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

SAPE-001 SOLUTION: Contains 1 mg/mL SAPE 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 0.09% sodium azide.

Store at refrigerator temperatures, 2-8^{o}C.

Protect from exposure to sunlight.

AVAILABLE SAPE DILUENT

PECD-100 SOLUTION: Contains a proprietary phosphate buffered saline solution, pH 7.3 with 0.09% sodium azide preservative.

Store at refrigerator temperatures, 2-8^{o}C.

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QC USING FRET METHOD

The Moss Inc. FRET test was developed for internal use to test SAPE conjugates, although of course any suitable methodology may be used to test SAPE conjugates. SAPE serves as the energy transfer donor in the test. The energy transfer acceptor conjugate is biotinylated-allophycocyanin (BAPC) 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 (SAPE and buffer in Rows A and B). Each column also has n=2 donor plus acceptor (SAPE and BAPC in Rows C and D). One or more columns can be used for the control with no donor.

1. Prepare 0.01 mg/mL dilutions of SAPE and BAPC using buffer 10 mM PBS, pH 7.3, 1.5 mg/mL BSA. It is convenient to use a second microtiter plate to prepare the SAPE 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 SAPE donor conjugates to each well in rows A, B, C and D. A different SAPE donor conjugate can be used for each column, and in this case n=2. We usually use the same SAPE conjugate for 4 columns, so n=8 for in this case.
4. To the lower half of the plate, add 50 µL of the next set of SAPE donor conjugates to each well in rows E, F, G and H.
5. Add 50 µL of BAPC 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 SAPE conjugate, for the donor alone (Fo), and for the donor plus acceptor (F). In our hands, the CVs are generally 1-3%.
2. Subtract the readings for the buffer alone control from Fo, and subtract the fluorescence readings for buffer plus acceptor control from F.
3. Divide F/Fo values that were corrected for the background control. 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 SAPE 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.