

Anti-human EGFR aptamer (clone 2369), FITC Conjugate

Description

AptSci AptoCyto™ provides dye-conjugated aptamer for Flow Cytometry which sufficient to perform 100 tests.

Product Information

- **Product name:** FITC-conjugated monoclonal anti-human EGFR aptamer (clone 2369)
- **Content:** FITC-conjugated monoclonal anti-human EGFR aptamer required to perform 100 tests
- **Catalog number:** 2369FC-FITC
- **Protein source for generation of aptamer:**
Recombinant protein produced in mammalian cells
- **Specificity:** Anti-EGFR aptamer binds to human EGFR. Cross reactivity with other species has not been tested.
- **MW:** 17.3 kDa
- **Tested applications:** FACS
- **Form:** Dried form.
- **Shipping:** At ambient temperature.

Storage

Dye-conjugated aptamers are stable for 12 months from date of receipt when stored in the dark at 2°C-8°C.

Additional Reagents Required

PBS buffer including 5mM MgCl₂ (Binding buffer)

Intended Use

Designed to quantitatively determine the percentage of cells Bearing EGFR within a population and qualitatively determine the density of EGFR on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the FITC-labeled monoclonal aptamer, which binds to cells expressing EGFR. Unbound FITC-conjugated aptamer is then washed from the cells. Cells expressing EGFR are fluorescently stained, with the intensity of staining directly proportional to the density of EGFR. Cell surface expression of EGFR is determined by flow cytometric analysis using 494 nm wavelength laser excitation.

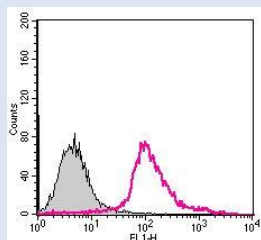


Fig. 1. Flow cytometry histograms showing the binding of representative EGFR aptamer against the target A431 cells. Approximately 1×10^6 cells were washed and incubated with FITC-conjugated EGFR aptamer (Pink histogram). The untreated cell was used as background fluorescence signal (Gray histogram).

Cell Preparation

Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed two times in an isotonic PBS buffer (Ca²⁺, Mg²⁺ free), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in binding buffer to a final concentration of 1×10^7 cells/mL.

Note: Adherent cell lines may require pretreatment with Trypsin/EDTA(TE) or 5 mM EDTA to facilitate removal from substrate. We recommend that cells are detached by short time processing using TE in order not to affect membrane protein and sieved with a nylon mesh of 40 µm pore size prior to sorting to remove clumps. Detached cells should be washed two times in an isotonic PBS buffer (Ca²⁺, Mg²⁺ free).

Sample Staining

- 1) Transfer 100 µL of cells (1×10^6) per assay into a 1.5 mL tube for staining.
- 2) Perform heating & cooling step for the regeneration of aptamer. 20 pmol aptamers are diluted in 20 µL of binding buffer and heated 5 minutes at 95°C followed by slowly cooled at room temperature. Slow cooling means keep the tubes on the bench for 15 min.
- Note : Protect from light during process.
- 3) Add 20 pmol of FITC-conjugated anti-EGFR aptamer.
- 4) Incubate for 15-20 minutes at 2°-8° C in the rocking mixer.
- 5) Following this incubation, remove unreacted anti-EGFR aptamer by centrifuge and wash the cells twice in 1 mL of PBS buffer (Ca²⁺, Mg²⁺ free).
- 6) Resuspend the cells in 200-500 µL of PBS buffer for final flow cytometric analysis.

Note: As a control for analysis, FITC-conjugated control aptamers are available.

LIMITATIONS

Warranty: AptSci AptoCyto™ products are warranted to meet stated product specifications and to confirm to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sales for products used, handled and stored according to AptSci's instructions. AptSci's sole liability is limited to replacement of the product or refund of the purchase price. AptoCyto™ products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. AptoCyto™ products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from AptSci.



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