

Product Name: Ep23, 5'-DY647**Cat. No. 6102**

Binding data

Ep23, 5'-DY647 has a K_d of 39.42 nM against EpCAM as measured by flow cytometry (Figure 1).

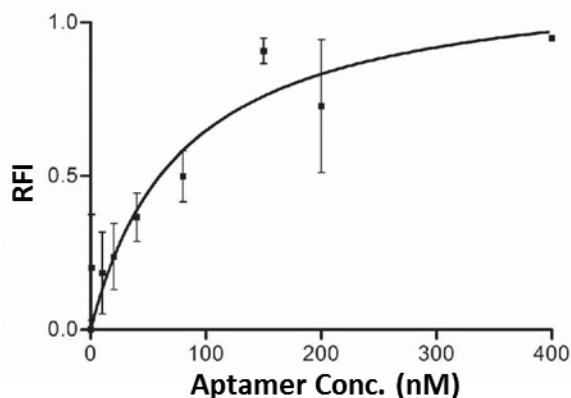


Figure 1. Binding curve data (flow cytometry) for Ep23, 5'-DY647 aptamer on HT29 cells

Reconstitution and refolding protocol

Reconstitute the aptamer at 100 μ M in sterile deionized H₂O and ensure the aptamer has fully dissolved. We recommend that stock solutions, once prepared, are stored aliquoted in tightly sealed vials at -20°C or below and used within 1 month. Wherever possible solutions should be made up and used on the same day. Repeated freeze thaw cycles should be avoided.

Prior to use, dilute the aptamer at an assay dependent concentration in PBS buffer containing 5 mM MgCl₂. Heat the solution at 85°C for 5 min, incubate for 10 min at room temperature and finally allow to refold for 15 min at 37°C.

Unfixed cell imaging protocol (this protocol is also applicable to flow cytometry)

Twenty-four hours prior to labelling, seed cells at a density of 75,000 cells per cm² in an 8-chamber slide (Lab-Tek II, Nunc). Remove media and incubate cells in blocking buffer (PBS buffer containing 5 mM MgCl₂, 0.1 mg/mL tRNA (R5636, Sigma), and 5% FBS) at 37 °C for 15 min. Wash twice in binding buffer (DPBS with 5 mM MgCl₂, 0.1 mg/mL tRNA and 0.1 mg/mL salmon sperm DNA) prior to incubation with 200 nM (1/500 dilution) refolded aptamer (typically 100 μ L) for 30 min at 37 °C. Additional step for unfixed cell imaging only: add Hoechst 33342 (3 μ g/mL) to the cells during the final 15 min of incubation. Remove the aptamer solution and wash three times for 5 min each in binding buffer prior to visualization.

Reagent	Tocris catalog number
PBS	5564
Hoechst 33342	5117

Fixed tissue imaging protocol

Deparaffinize paraffin embedded sections with Histoclear and rehydrate through graded ethanols. Perform heat induced antigen retrieval in a microwave oven using Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, 0.05% Tween® 20, pH 9.0) for 20 min and allow slides to cool prior to blocking with 0.1 mg/mL tRNA (R5636, Sigma), and 1 mg/mL bovine serum albumin or 10% goat serum in phosphate buffered saline (PBS) for 1 h. Following the blocking step, wash slides in PBS containing 0.1% Tween® 20 twice for 2 min prior to incubation with aptamer. Refold Ep23, 5'-DY647 using the refolding procedure above, and apply to tissue at a concentration of 100 nM in PBS containing 5 mM MgCl₂, 0.1 mg/mL tRNA, 1 mg/mL BSA, 10% dextran sulfate and 500 mg/mL heparin for 15 min at 37°C. Wash slides in PBS containing 0.1% Tween® 20 three times for 5 min each prior to incubation with Hoechst 33342 (3 µg/mL) for 10 min. Mount slides using VECTASHIELDH (Vector Laboratories, Burlingame, CA) and apply coverslips. N.B: The aptamer must be refolded according to the above procedure prior to the addition of 0.1 mg/mL tRNA, 1 mg/mL BSA, 10% dextran sulfate and 500 mg/mL heparin.

This protocol has successfully been used for IHC EpCAM staining in the following xenograft tumors: T47D, MCF7, MDA-MB-231 (breast cancer) and HT-29 (colon cancer).

Reagent	Tocris catalog number
Tris-HCl	3164
EDTA	2811
BSA	5217
PBS	5564
Heparin	2812
Hoechst 33342	5117

Tween is a registered trademark of ICI Americas.

Unfixed tumorspheres imaging protocol

Wash spheres three times in PBS containing 5 mM MgCl₂ and block for 20 min using blocking buffer (PBS buffer containing 5 mM MgCl₂, 0.1 mg/mL tRNA and 5% FBS). Incubate spheres with 100 nM refolded Ep23, 5'-DY647 for a minimum of 30min. Wash spheres three times with PBS prior to visualization.

This protocol has been produced in collaboration with Dr Sarah Shigdar, Deakin University, Australia
Further information is available in the following publications:

Shigdar et al (2011) RNA aptamer against a cancer stem cell marker epithelial cell adhesion molecule. *Cancer Sci.* **102** 991. PMID: 21281402.

Shigdar *et al* (2013) The use of sensitive chemical antibodies for diagnosis: detection of low levels of Epcam in breast cancer. *PloSOne*. **8** e57613.

Xiang *et al* (2015) Superior performance of aptamer in tumor penetration over antibody: implication of aptamer-based theranostics in solid tumors. *Theranostics*. **5** 1083. PMID: 26199647.