Product Name: CD133-A15, 5'-DY647 **Cat. No. 6103**

Binding data

CD133-A15, 5'-DY647 has a K_d of 33.9 nM (Hep3B cells), 83.2 nM (HT-29 cells) against CD133 as measured by flow cytometry (Figure 1).

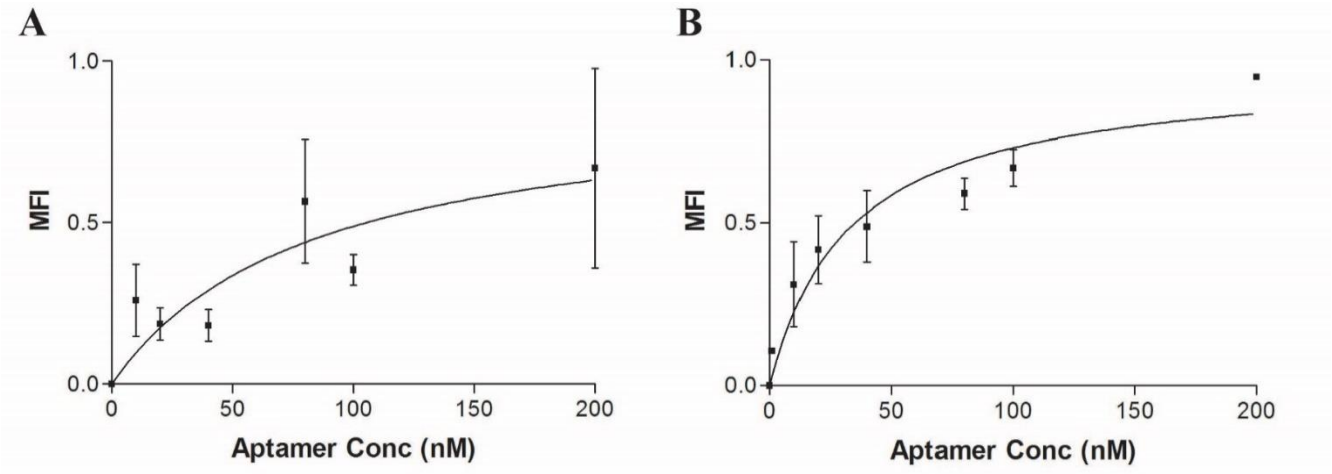

Figure 1. Binding curve data (flow cytometry) for CD133-A15, 5'-DY647 aptamer on (A) HT-29 cells and (B) Hep3B cells.

Reconstitution and refolding protocol

Reconstitute the aptamer at 100 µM in sterile dH_2O 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 2.5 mM $MgCl_2$. 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 cm^2 in an 8-chamber slide (Lab-Tek II, Nunc). Remove media and incubate cells in blocking buffer (PBS buffer containing 2.5 mM $MgCl_2$, 0.1 mg/mL tRNA (R5636, Sigma), and 5% FBS) at 37 ˚C for 15 min. Wash twice in binding buffer (DPBS with 2.5 mM $MgCl_2$, 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 µL) for 30 min at 37 ˚C. Additional step for unfixed cell imaging only: add Hoechst 33342 (3 µg/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

Imaging in unfixed tumorspheres protocol

Wash spheres three times in PBS containing 2.5 mM $MgCl_2$ and block for 20 min using blocking buffer (PBS buffer containing 2.5 mM $MgCl_2$, 0.1 mg/mL tRNA and 5% FBS). Incubate spheres with 100 nM (1/1000 dilution) refolded aptamer 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 publication:

Shigdar *et al* (2013) RNA aptamers targeting cancer stem cell marker CD133. Cancer Lett. ***300*** 84. PMID: 23196060.