Store at 4°C

# Posibeads™ Whitlow/218 Linker Positive Control Beads

#26254

250 µL

Cell Signaling TECHNOLOGY®

**Support:** +1-978-867-2388 (U.S.)
cellsignal.com/support

**Orders:** 877-616-2355 (U.S.)
orders@cellsignal.com

**For Research Use Only. Not for Use in Diagnostic Procedures.**

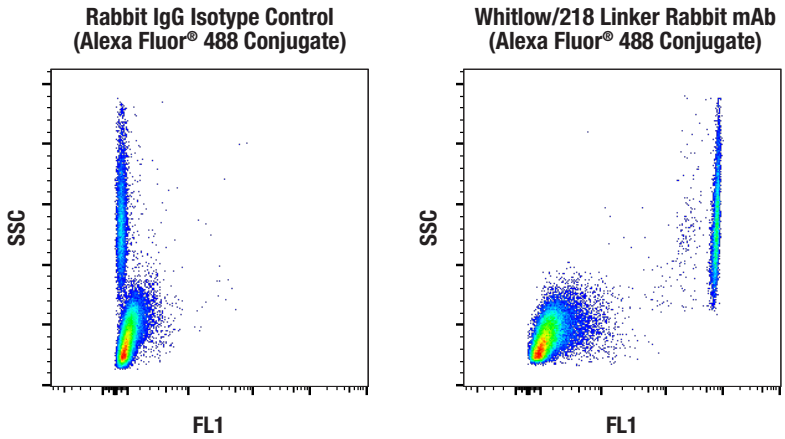
**Description:** Posibeads™ provide a unique positive control to confirm specific binding and fluorescence of your antibody conjugate of interest. Posibeads™ behave like cells in suspension, and can be carried through immunostaining and wash steps following the same procedure as your cell-based samples. The 5 nm non-fluorescent polystyrene beads are coated with Whitlow/218 linker peptide. When incubated in the presence of a fluorescent antibody conjugate targeting Whitlow/218 linker, the antibody will bind to the beads and result in increased fluorescence relative to unlabeled beads. Unlike compensation beads, the binding is driven by the antibody specifically targeting its antigen. Because of this, these Posibeads™ can be used as a specific control for a Whitlow/218 linker antibody even when tested as part of a panel of other conjugated antibodies.

Posibeads™ are reproducibly generated and can also be used as a control to enable comparison of fluorescence across multiple instruments, sites, timepoints, and experiments. They can be used as a separate standalone sample or added to a sample of interest to serve as an internal positive control verifying the addition and functional activity of the conjugated antibody. Posibeads™ are not known to bind to any cell types, but a lower number of beads is recommended when mixing with cells to reduce the likelihood of acquiring concurrent or overlapping cell and bead events.

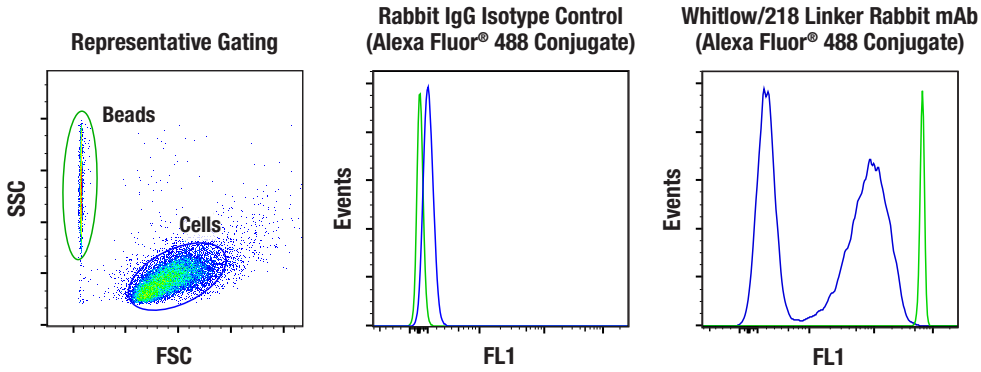
**Directions for Use:** Posibeads™ can be used as an independent positive control sample or mixed with cells to function as an internal positive control. Recommended usage is 5 µL (around 100,000 beads) for use as an independent sample or 1 µL when mixed with cells. (If desired, perform a titration to determine the optimal ratio of beads to cells for your experiment.)

1. Prepare cells/tissue in tubes or wells as necessary for your experiment. If Posibeads™ will be used as an independent positive control, add an equivalent volume of buffer to empty tube(s) or well(s) as desired.
   **NOTE:** Plan to include at least one unstained control to establish background fluorescence in your channel(s) of interest.
2. Mix or vortex the Posibeads™ immediately prior to use to ensure the beads are evenly distributed in solution.
3. Add desired volume of Posibeads™ to the control tube(s) or well(s), or add directly into the cell samples for use as an internal control.
4. Proceed with immunostaining following established protocols. The same incubation and wash steps should be performed with samples of interest and the Posibead control samples. The same centrifugation conditions may be used for both Posibeads™ and your cells of interest.
5. Acquire samples on flow cytometer. In mixed samples, beads will be differentiated from cells by high side scatter and low forward scatter.

**Storage:** Store at 4ºC. Supplied in 1X PBS (pH 7.4), 0.05% Tween 20, and 0.05% sodium azide.




*Whitlow/218 Linker Posibeads™ were added to live Fc-blocked PBMCs. The cells and Posibeads™ were then immunostained with either Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 488 Conjugate) #2975 (left) or Whitlow/218 Linker (E3U7Q) Rabbit mAb (Alexa Fluor® 488 Conjugate) #55809 (right). Fluorescence data (FL1) are plotted versus side scatter (SSC) to enable differentiation of cells (low SSC) and beads (high SSC).*




*Whitlow/218 Linker Posibeads™ were added to a mixed population of live cells containing wild-type Jurkat cells and Jurkat cells engineered to express an scFv-based Anti-CD19 CAR containing a Whitlow/218 linker. The cells and Posibeads™ were then immunostained with either Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 488 Conjugate) #2975 (center) or Whitlow/218 Linker (E3U7Q) Rabbit mAb (Alexa Fluor® 488 Conjugate) #55809 (right). Cells and beads were differentiated by forward scatter (FSC) and side scatter (SSC) as shown (left), and fluorescence data (FL1) are displayed for cells (blue) and Posibeads™ (green) for both immunostaining conditions. CAR cell line was provided by the Lohmueller Lab, University of Pittsburgh.*







**Applications:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
**Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
**All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.