

Technical Data Sheet

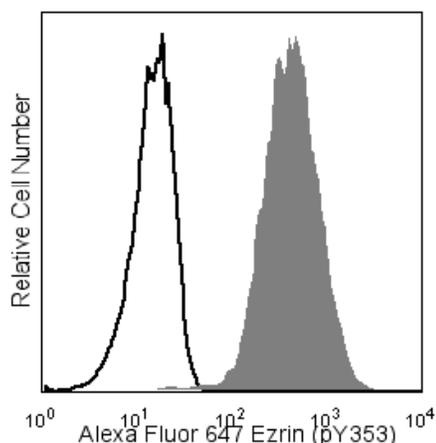
Alexa Fluor® 647 Mouse anti-Ezrin (pY353)**Product Information**

Material Number:	558628
Size:	50 tests
Vol. per Test:	20 µl
Clone:	I66-386
Immunogen:	Phosphorylated Human Ezrin Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Ezrin is a member of the ERM (ezrin-radixin-moesin) family of proteins that function as crosslinkers between the actin cytoskeleton and the plasma membrane. Ezrin is a substrate for the tyrosine kinase epidermal (EGF) and hepatocyte growth factor (HGF) receptors. Upon EGF or HGF stimulation, ezrin is phosphorylated on tyrosine 353 (Y353), which is required for its interaction with phosphatidylinositol 3 kinase p85 subunit and activation of Akt.

The I66-386 monoclonal antibody recognizes the phosphorylated Y353 of human ezrin.



Analysis of Ezrin (pY353) in human chronic myelogenous leukemia. Serum-starved K-562 cells (ATCC CCL-243) were either treated with 1 mM Pervanadate (Sigma Cat. No. S6508) at 37 °C for 20 minutes (shaded histogram) or untreated (open histogram). The cells were fixed (BD Cytotfix™ buffer, Cat. No. 554655) for 10 minutes at 37 °C, then permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-Ezrin (pY353). Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes**Application**

Intracellular staining (flow cytometry)	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

- This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100-µl experimental sample (a test).
- Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
- Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).

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4. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

- Gautreau A, Pouillet P, Louvard D, Arpin M. Ezrin, a plasma membrane–microfilament linker, signals cell survival through the phosphatidylinositol 3-kinase/Akt pathway. *Proc Natl Acad Sci U S A*. 1999; 96:7300-7305. (Biology)
- Krieg J, Hunter T. Identification of the two major epidermal growth factor-induced tyrosine phosphorylation sites in the microvillar core protein ezrin. *J Biol Chem*. 1992; 267(27):19258-19265. (Biology)