

Technical Data Sheet

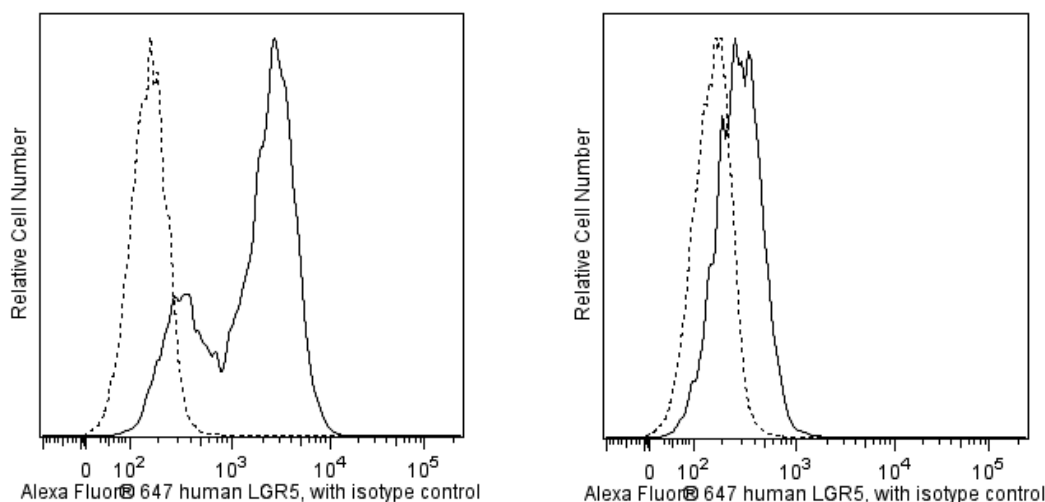
Alexa Fluor® 647 Rat anti-Human Lgr5 (Central LRR)**Product Information**

Material Number:	562912
Alternate Name:	GPR49, GPR67, HG38
Size:	50 tests
Vol. per Test:	5 µl
Clone:	4D11F8 (also known as 4D11)
Immunogen:	Human LGR5 DNA
Isotype:	Rat IgG2b, λ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

Lgr5 (leucine-rich-repeat-containing G-protein-coupled receptor 5) is a seven transmembrane-domain receptor that is a target gene for Wnt and marks stem cells in the small intestine, colon, stomach, and hair follicle. Lgr5 was initially identified as a potential stem cell marker due to restricted expression of Lgr5 in the intestinal crypt and labeling of rapidly cycling cells of the colon and intestine. Using both lineage tracing and organoid culture experiments, Lgr5 positive cells are capable of generating all types of the small intestine epithelium hence indicating that Lgr5 marks stem cells of the small intestine and colon. R-spondin growth factors, which are secreted agonists of the Wnt pathway, bind Lgr5. The binding of R-spondins to Lgr5 leads to recruitment of the Frizzled/LRP Wnt receptor complex, which binds to Wnt ligands and leads to downstream Wnt signaling. Lgr5 is up-regulated in colon and ovarian cancers and has been implicated in promotion of tumor growth and metastasis.

The 4D11F8 monoclonal antibody recognizes an epitope in the center of the leucine-rich repeat (LRR) region of Human Lgr5.



Flow cytometric analysis of LGR5 expression on human LGR5-transfected cells. LS 174T colorectal adenocarcinoma cells transfected with human LGR5 (cells from Dr. Hans Clevers, Hubrecht Institute, Left Panel) and parental LS 174T cells, which express low levels of endogenous LGR5 (ATCC CL-188, Right Panel) were harvested using Accutase™ Cell Detachment Solution (Cat. No. 561527). The cells were stained with either Alexa Fluor® 647 Rat IgG2b, κ Isotype Control (Cat. No. 557691; dashed line histogram) or Alexa Fluor® 647 Rat anti-Human Lgr5 (Central LRR) antibody (Cat. No. 562912; solid line histogram) at matched concentrations. The fluorescence histograms were derived from gated events based on the light scattering characteristics of viable LS 174T cells. Flow cytometry was performed on a BD FACSCanto™ II Flow Cytometry System.

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Preparation and Storage

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.

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

Application Notes

Application

Flow cytometry	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
557691	Alexa Fluor® 647 Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
561527	Accutase™ Cell Detachment Solution	100 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100- μ l experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.
6. 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.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

Barker N, Huch M, Kujala P, et al. Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell*. 2010; 6(1):25-36. (Biology)

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Carmon KS, Gong X, Lin Q, Thomas A, Liu Q. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/[beta]-catenin signaling. *Proc Natl Acad Sci U S A*. 2011; 108(28):11452-11457. (Biology)

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