Technical Data Sheet

BV480 Goat Anti-Rat Ig

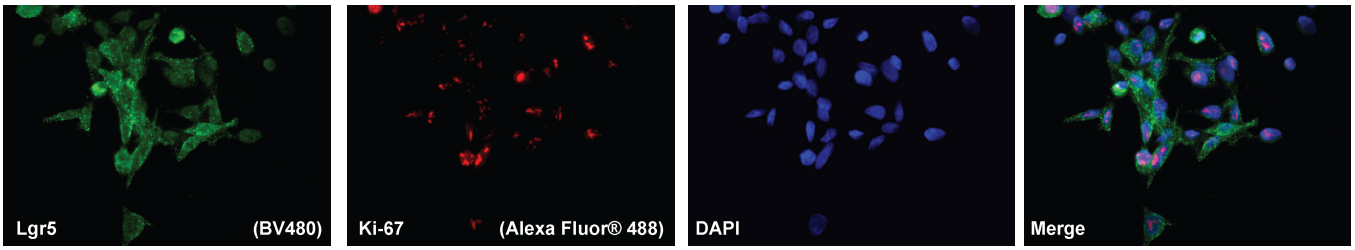
Product Information

Material Number:	**564878**
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	Polyclonal
Isotype:	Goat Ig
Reactivity:	QC Testing: Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD Horizon™ BV480 Goat Anti-Rat Ig is intended to be a second-step reagent for immunofluorescent staining of cells pre-stained with Rat Ig primary antibodies. It also stains rat B cells and has little reactivity with rat non-B cells or mouse splenocytes. As a second step, it is reactive with rat IgG and IgM monoclonal antibodies although a weaker signal may be detected when the primary antibody has a rat IgM or IgG2b isotype due to its adsorption with mouse Ig. It has weak cross-reactivity detectable by flow cytometry with some, but not all, hamster immunoglobulins.

The antibody was conjugated to BD Horizon BV480 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max of 436-nm and Em Max at 478-nm, BD Horizon BV480 can be excited by the violet laser and detected in the BD Horizon BV510 (525/40-nm) filter set. BV480 has less spillover into the BV605 detector and, in general, is brighter than BV510.



Immunofluorescence staining of human LS174T cells. *Colorectal adenocarcinoma LS174T cells transfected with human LGR5 (cells from Dr. Hans Clevers, Hubrecht Institute) were cultured and fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), blocked with 5% Goat serum and 1% BSA diluted in 1x PBS, and stained with Purified Rat Anti-Human Lgr5 (Central LRR) antibody (Cat. No. 562732) at 2.5 µg/ml. After washing, the cells were stained with the second step reagent, BD Horizon™ BV480 Goat Anti-Rat Ig (Cat. No. 564878) (pseudo-colored green) at 2.5 µg/mL. Cells were then washed and permeabilized with 0.1% Triton™ diluted in 1x PBS. Cells were then stained with Alexa Fluor® 488 Mouse Anti-Human Ki-67 antibody (Cat No. 558616) (pseudo-colored red) in blocking buffer with 5% Goat serum, 1% BSA, and 0.5% Triton™ diluted in 1x PBS. DAPI (Cat. No. 564907) was used as a nuclear counterstain (pseudo-colored blue). Slides were mounted with ProLong® Gold and the image was captured on a BD Pathway™ 435 Cell Analyzer (epifluorescence microscope) and merged using BD Attovision™ Software. 40X objective.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The polyclonal antibody was purified from antiserum by negative adsorption and affinity chromatography.
The antibody was conjugated with BD Horizon BV480 under optimum conditions, and unconjugated antibody and free BD Horizon BV480 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested
Immunofluorescence	Tested During Development

Recommended Assay Procedure:

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349).

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For Immunofluorescence Applications:
The use of a mounting reagent (eg, ProLong® Gold) is highly recommended to maximize the photostability of BV480. For confocal microscopy systems, a 440 nm laser is the optimal excitation source and the recommended emission filter is a 485/20 nm bandpass filter.

For epifluorescence microscopes with broad spectrum excitation sources, the recommended excitation and emission filters are 445/20 nm and 485/20 nm bandpass filters, respectively. For specific multicolor imaging applications, the exact filter configurations should be optimized by the end user. For additional instrument/filter configuration information, please visit http://www.bdbiosciences.com/research/cellularimaging.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 mL	(none)
562732	Purified Rat anti-Human Lgr5 (Central LRR)	0.1 mg	4D11F8
558616	Alexa Fluor® 488 Mouse anti-Ki-67	100 Tests	B56
564907	DAPI Solution	1 mg	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. 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.
4. BD Horizon Brilliant Violet 480 is covered by one or more of the following US patents: 8,575,303; 8,354,239.
5. Triton is a trademark of the Dow Chemical Company.
6. ProLong® is a registered trademark of Thermo Fisher Scientific, Inc. Waltham, MA.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
10. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.