# Cobalt BIOLOGICS

# Anti-rabbit CD19

Date : 2020.12.12

# Anti-Rabbit CD19

## Overview

**Description:** Recombinant monoclonal to rabbit CD19
**Original host species:** Mouse
**Tested applications:** Flow Cyt, ELISA, BLI
**Species reactivity:** Rabbit

**Immunogen**
Recombinant rabbit  CD19 extracellular domain as defined by Uniprot Entry G1TWR4_RABIT, corresponding to amino acids Met1-Gly278

**Molecular cloning**
Immunoglobulin light and heavy variable domains obtained from a mouse hybridoma by RT-PCR. Reassembled as full recombinant mouse IgG1 and binding confirmed on purified rabbit CD19 extracellular domain by ELISA, and flow cytometry on both HEK293 expressing rabbit CD19 and native rabbit lymphocyte cells
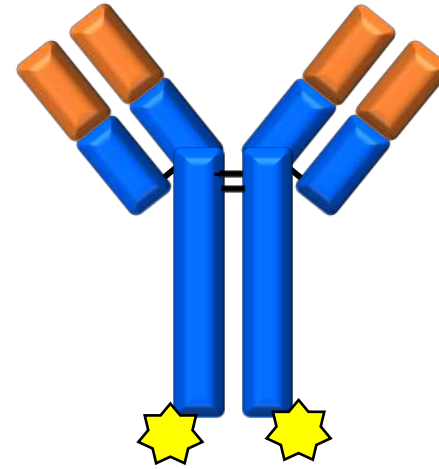
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# Available Formats
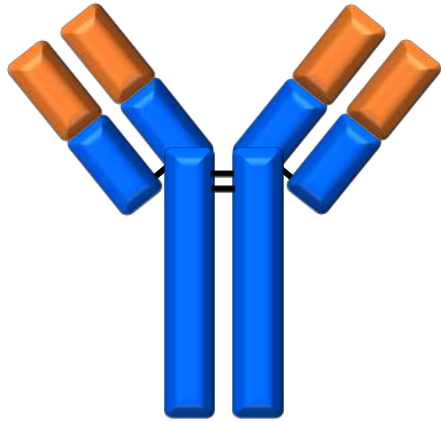
Mouse IgG2a
with NullFc™
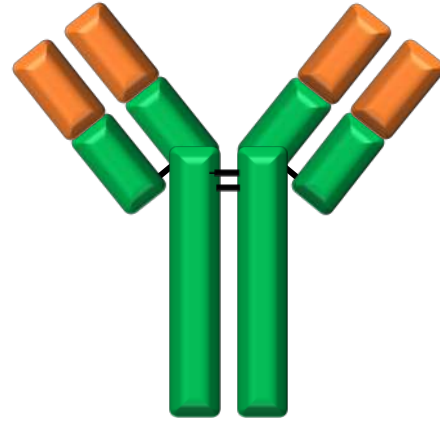
Human IgG1
with NullFc™

Mouse IgG2a NullFc™
with site-specific
biotinylation

- Recombinantly produced in three formats:
    - Mouse IgG2a NullFc™ (NullFc detailed in later slide)
    - Human IgG1 NullFc™
    - Mouse IgG2a NullFc™ site-specific biotinylation at C-terminus of heavy chain

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# Characterization

**Flow Cytometry with Rabbit Lymphocytes**
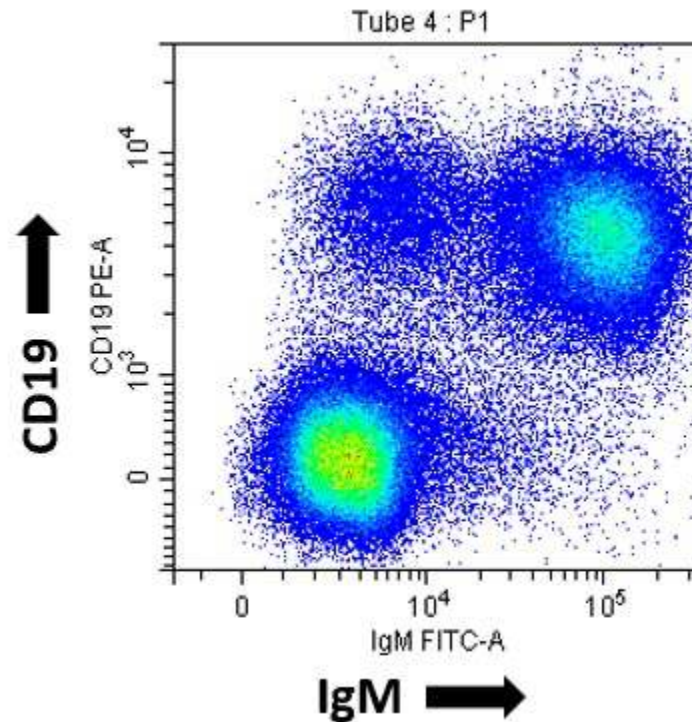


*Rabbit PBMCs prepared from rabbit whole blood with Lympholyte Rabbit (Cedarlane #CL5050)*

Anti-rabbit CD19 human IgG1.
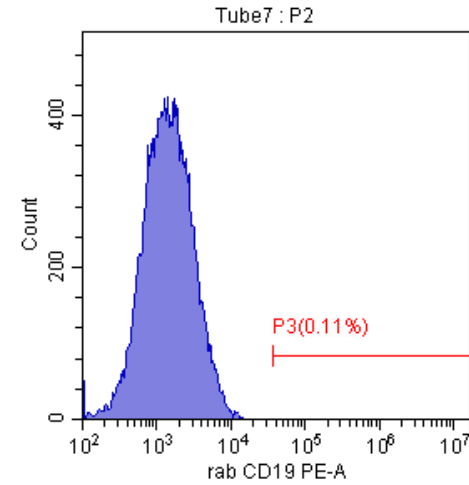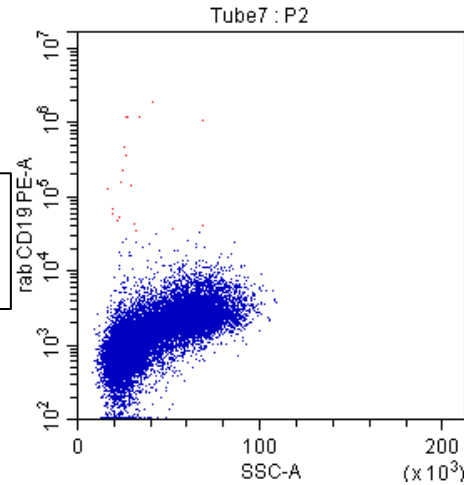Secondary: goat anti-human IgG-PE +
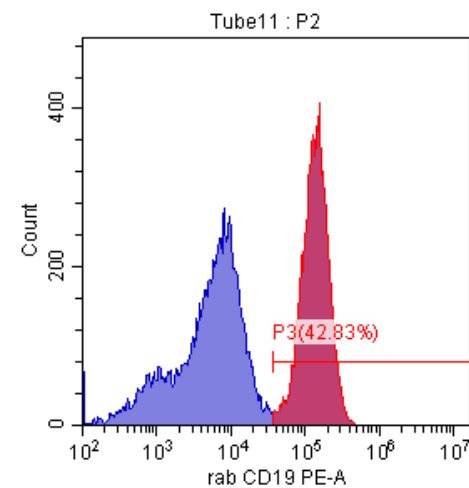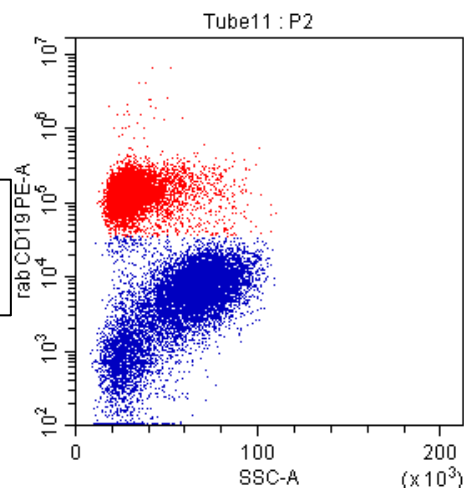goat anti-rabbit IgM-FITC

# Characterization

## Flow Cytometry with Rabbit Lymphocytes

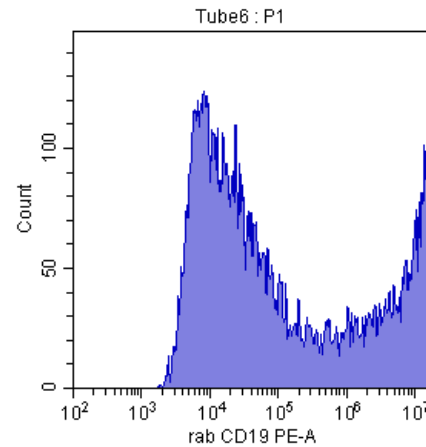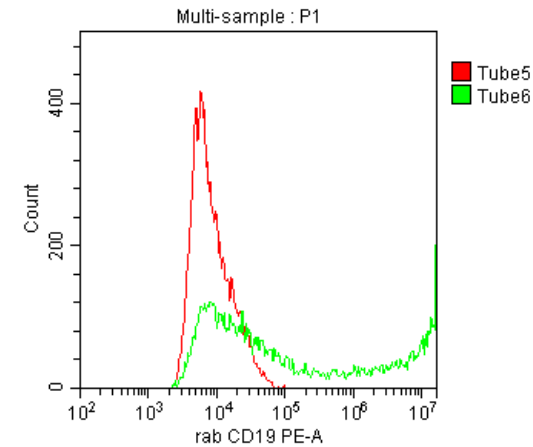# Characterization

## Flow Cytometry with 293-Expressed Rabbit CD19
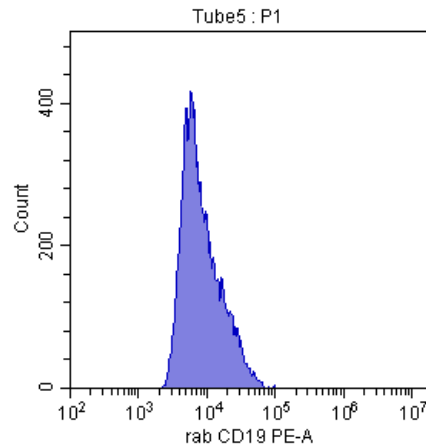


Mock-transfected
293 cells

Rabbit CD19
transfected 293 cells

Histogram overlay

- HEK293 cells were transfected with full-length rabbit CD19, or mock transfected with no DNA (negative control)
- Stained with anti-rabbit CD19 human IgG1 and anti-human IgG-PE secondary
- Robust signal seen in CD19-transfected cells by flow cytometry

# Characterization

**Species Cross-Reactivity**
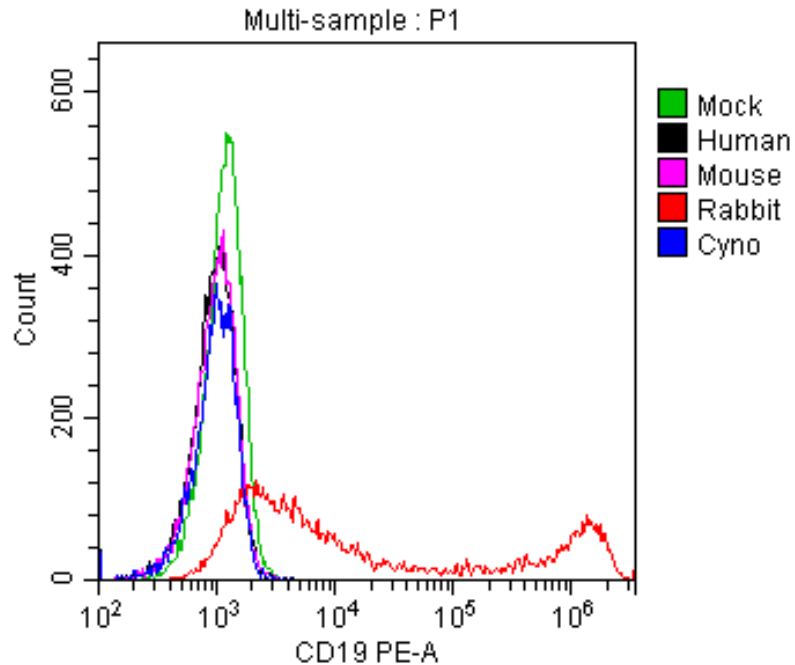


- Flow cytometry on HEK293 cells with cell surface expression of:
  - No DNA (Mock)
  - Human CD19
  - Mouse CD19
  - Rabbit CD19
  - Non-human primate CD19 (Cyno)

- Antibody only binds to rabbit CD19 and not to the other species tested

# Characterization

## Cell Depletion

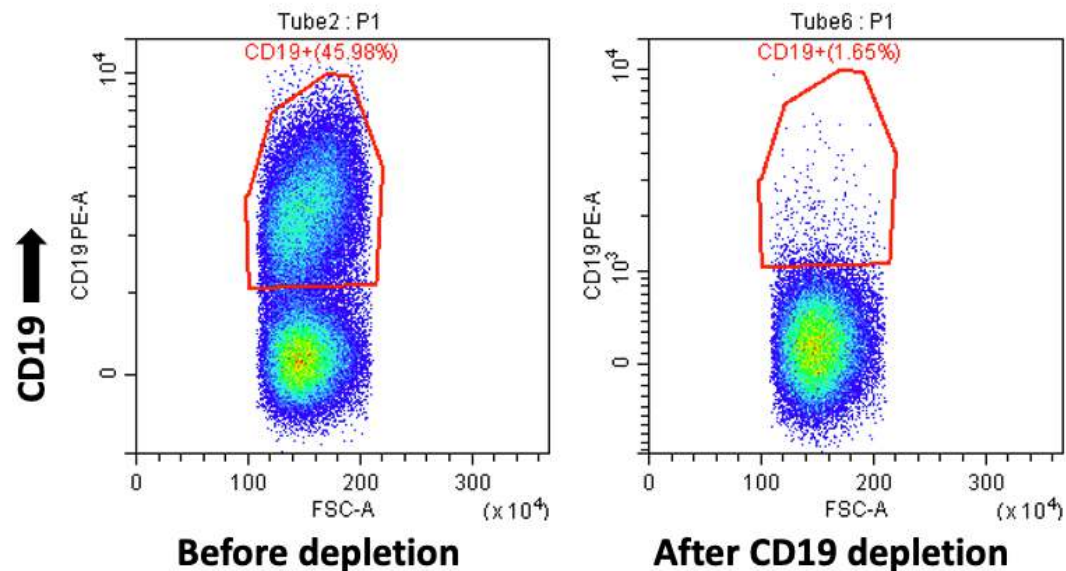- The biotinylated anti-CD19 antibody can be used to enrich or deplete CD19+ rabbit cells using a streptavidin matrix



**Before depletion**     **After CD19 depletion**

*Rabbit PBMCs prepared from rabbit whole blood with Lympholyte Rabbit (Cedarlane #CL5050)*
*CD19+ cells were depleted using anti-rabbit CD19-biotin (Cobalt cat.# 200103) along with Dynabeads™ M-280 Streptavidin from Thermo Fisher Scientific (cat.# 11205D). Depletion protocol available on antibody product page at www.cobaltbiologics.com*

# NullFc™



**msIgG2a WT Fc**          **msIgG2a NullFc**
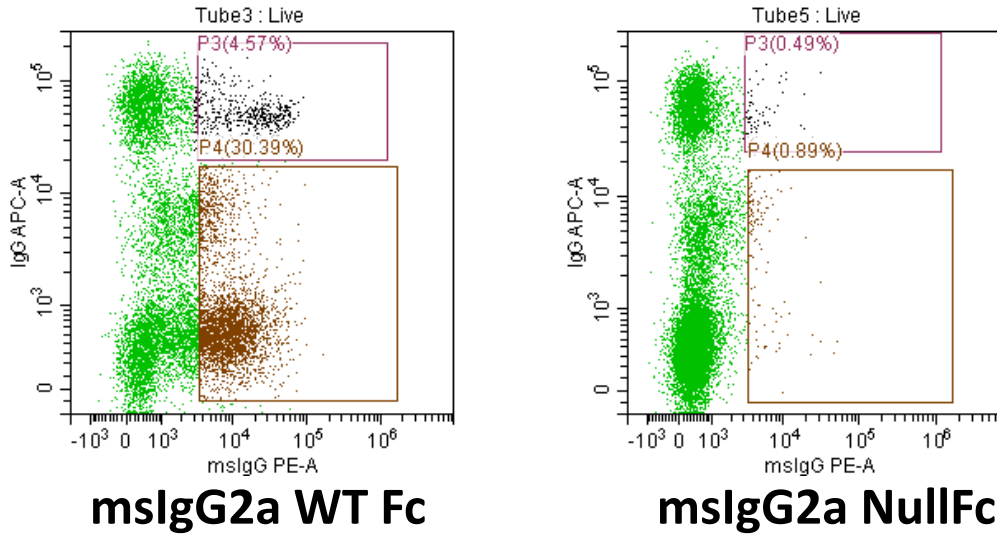
WT Fc background: 35%
NullFc™ background: 1.4%

*Rabbit lymphocytes prepared from rabbit whole blood with Lympholyte Rabbit (Cedarlane #CL5050)*

- The Fc domain of mouse IgG2a binding to rabbit lymphocytes
  - Wild-type Fc on the left, NullFc™ on the right
- NullFc™ is an engineered Fc with point mutations to reduce binding to Fc receptors and thus reduce background binding
- There is significant background binding of the wild-type mouse IgG2a Fc, presumably via the rabbit Fc receptors
- NullFc™ significantly reduces the background binding of mouse IgG2a-Fc
- NullFc™ is compatible with traditional secondary detection reagents

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# Characterization

## SDS-PAGE
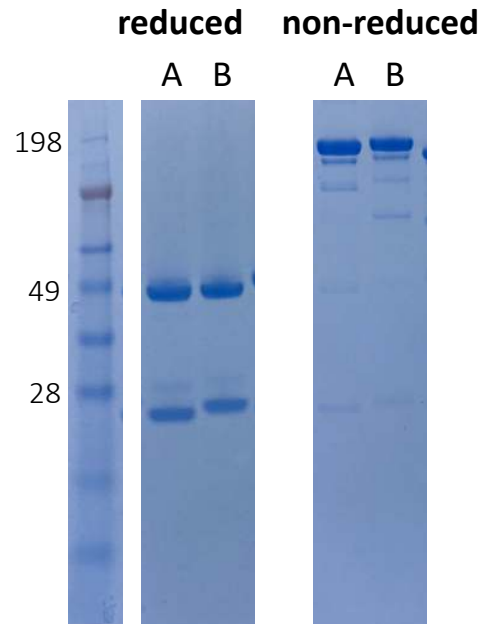


A: Anti-rabbit CD19 mouse IgG2a NullFc™
B: Anti-rabbit CD19 human IgG1 NullFc™

- SDS-PAGE analysis of purified antibodies
- Reduced and non-reduced
- Showing >95% purity

# Products

Currently Available:

Cat. # 200101 – Anti-rabbit CD19 mouse IgG2a NullFc™
Cat. # 200102 – Anti-rabbit CD19 human IgG1 NullFc™
Cat. # 200103 – Anti-rabbit CD19 mouse IgG2a NullFc™
 with site-specific biotinylation

Visit www.cobaltbiologics.com



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