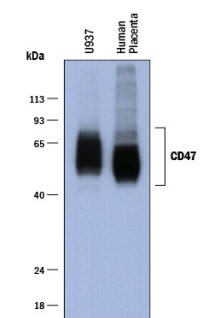
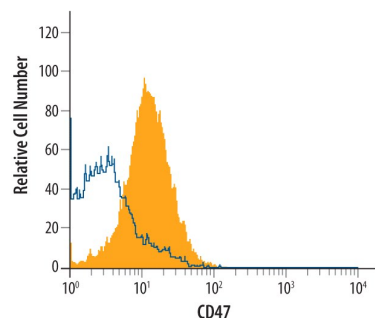
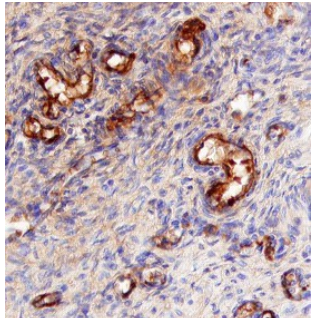
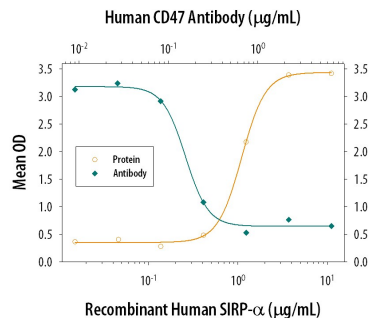
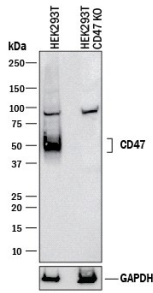


DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human CD47 in direct ELISAs and Western blots. In direct ELISAs and Western blots, less than 5% cross-reactivity with recombinant mouse CD47 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human CD47 Gln19-Pro139 Accession # Q08722
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS									
Please Note: Optimal dilutions should be determined by each laboratory for each application. <i>General Protocols</i> are available in the <i>Technical Information</i> section on our website.									
	<table border="1"> <thead> <tr> <th>Recommended Concentration</th> <th>Sample</th> </tr> </thead> <tbody> <tr> <td>1 µg/mL</td> <td>See Below</td> </tr> <tr> <td>2.5 µg/10⁶ cells</td> <td>See Below</td> </tr> <tr> <td>5-15 µg/mL</td> <td>See Below</td> </tr> </tbody> </table>	Recommended Concentration	Sample	1 µg/mL	See Below	2.5 µg/10 ⁶ cells	See Below	5-15 µg/mL	See Below
Recommended Concentration	Sample								
1 µg/mL	See Below								
2.5 µg/10 ⁶ cells	See Below								
5-15 µg/mL	See Below								
Western Blot	1 µg/mL								
Flow Cytometry	2.5 µg/10 ⁶ cells								
Immunohistochemistry	5-15 µg/mL								
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.								
Knockout Validated	CD47 is specifically detected in HEK293T human embryonic kidney parental cell line but is not detectable in CD47 knockout HEK293T cell line.								
Neutralization	Measured by its ability to neutralize SIRPα/CD172a-mediated adhesion of human erythrocytes. The adhesion of human erythrocytes to immobilized Recombinant Human SIRPα/CD172a/Fc Chimera (2 µg/mL, 100 µL/well) was maximally inhibited (70-100%) by 2 µg/mL of the antibody.								

DATA	
<p>Western Blot</p>  <p>Detection of Human CD47 by Western Blot. Western blot shows lysates of U937 human histiocytic lymphoma cell line and human placenta tissue, not heated to minimize aggregation. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human CD47 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4670) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). Specific bands were detected for CD47 at approximately 45-70 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Flow Cytometry</p>  <p>Detection of CD47 in Human Lymphocytes by Flow Cytometry. Human whole blood lymphocytes were stained with Sheep Anti-Human CD47 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4670, filled histogram) or control antibody (Catalog # 5-001-A, open histogram), followed by Northern-Lights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # NL010).</p>
<p>Immunohistochemistry</p>  <p>CD47 in Human Placenta. CD47 was detected in immersion fixed paraffin-embedded sections of human placenta using 5 µg/mL Sheep Anti-Human CD47 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4670) overnight at 4 °C. Tissue was stained with the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.</p>	<p>Neutralization</p>  <p>Cell Adhesion Mediated by SIRPα/CD172a and Neutralization by Human CD47 Antibody. Recombinant Human SIRPα/CD172a (Catalog # 4546-SA), immobilized onto a microplate, supports the adhesion of the human erythrocytes in a dose-dependent manner (orange line). Adhesion elicited by Recombinant Human SIRP-α (2 µg/mL) is neutralized (green line) by increasing concentrations of Sheep Anti-Human CD47 Polyclonal Antibody (Catalog # AF4670). The adhesion was maximally inhibited (70-100%) by 2 µg/mL of the antibody.</p>

Knockout Validated



Western Blot Shows Human CD47 Specificity by Using Knockout Cell Line. Western blot shows lysates of HEK293T human embryonic kidney parental cell line and CD47 knockout HEK293T cell line (KO). PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human CD47 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4670) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). Specific bands were detected for CD47 at approximately 50 kDa (as indicated) in the parental HEK293T cell line, but is not detectable in knockout HEK293T cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

CD47 (also integrin-associated protein/IAP and OA3) is a variably glycosylated, 40-60 kDa atypical member of the Ig-Superfamily. It is expressed on almost all cell types, including erythrocytes. CD47 binds to TSP-1 and SIRPα, and forms a membrane complex with CD36 and α_vβ₃. Mature human CD47 is a 305 amino acid (aa), five-transmembrane glycoprotein. It contains a 123 aa extracellular region (aa 19-141) that is characterized by the presence of a V-type Ig-like domain (aa 19-127), and a 34 aa C-terminal cytoplasmic tail that interacts with Giα subunits. Three splice variants occur over aa 293-323. Over aa 19-139, human CD47 shares 61%, 71% and 66% aa identity with mouse, porcine and canine CD47, respectively.