

DESCRIPTION

Species Reactivity	Human
Specificity	Detects recombinant human CD14 protein in Direct ELISA.
Source	Recombinant Monoclonal Rabbit IgG Clone # 3334A
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Synthetic peptide Accession # P08571
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose.

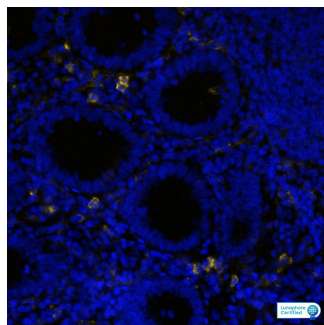
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. [General Protocols](#) are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Western Blot	2 µg/mL	Human tonsil
Multiplex Immunofluorescence	3 µg/mL	Immersion fixed paraffin-embedded sections of human appendix
Immunohistochemistry	1-10 µg/mL	Immersion fixed paraffin-embedded sections of human appendix and human liver

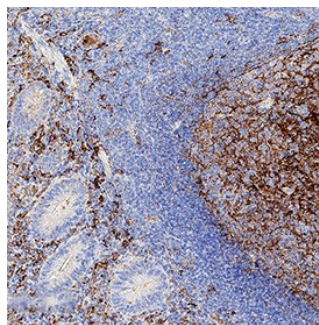
DATA

Multiplex Immunofluorescence



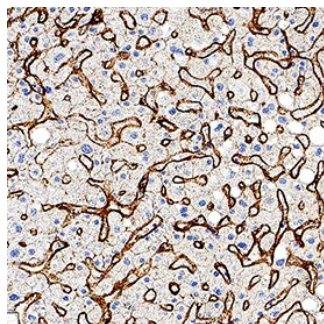
CD14 in Human Appendix via seqIF™ staining on COMET™
CD14 was detected in immersion fixed paraffin-embedded sections of human Appendix using Rabbit Anti-Human CD14, Monoclonal Antibody (Catalog #MAB11722) at 3µg/mL at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; Epredia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ Plus 647 Goat anti-Rabbit IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647RB) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the membrane. Protocol available in [COMET™ Panel Builder](#).

Immunohistochemistry



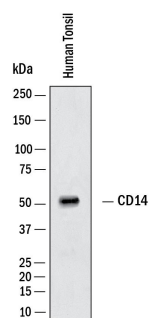
Detection of CD14 in Human Appendix. CD14 was detected in immersion fixed paraffin-embedded sections of human appendix using Rabbit Anti-Human CD14 Monoclonal Antibody (Catalog # MAB11722) at 3 µg/ml for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003) or the HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the membrane. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

Immunohistochemistry



Detection of CD14 in Human Liver. CD14 was detected in immersion fixed paraffin-embedded sections of human liver using Rabbit Anti-Human CD14 Monoclonal Antibody (Catalog # MAB11722) at 3 µg/ml for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003) or the HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the membrane. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

Western Blot



Detection of Human CD14 by Western Blot. Western Blot shows lysates of human tonsil. PVDF membrane was probed with 2 µg/ml of Rabbit Anti-Human CD14 Monoclonal Antibody (Catalog # MAB11722) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for CD14 at approximately 55 kDa (as indicated). This experiment was conducted under reducing conditions and using [Western Blot Buffer Group 1](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute lyophilized material at 0.2 mg/ml in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. • 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

CD14 is a 55 kDa cell surface glycoprotein that is preferentially expressed on monocytes/macrophages. The human CD14 cDNA encodes a 375 amino acid (aa) residue precursor protein with a 19 aa signal peptide and a C-terminal hydrophobic region characteristic for glycosylphosphatidylinositol (GPI)-anchored proteins. Human CD14 has four potential N-linked glycosylation sites and also bears O-linked carbohydrates. The amino acid sequence of human CD14 is approximately 65% identical with the mouse, rat, rabbit, and bovine proteins. CD14 is a pattern recognition receptor that binds lipopolysaccharides (LPS) and a variety of ligands derived from different microbial sources. The binding of CD14 with LPS is catalyzed by LPS-binding protein (LBP). The toll-like-receptors have also been implicated in the transduction of CD14-LPS signals. Similar to other GPI-anchored proteins, soluble CD14 can be released from the cell surface by phosphatidylinositol-specific phospholipase C. Soluble CD14 has been detected in serum and body fluids. High concentrations of soluble CD14 have been shown to inhibit LPS-mediated responses. However, soluble CD14 can also potentiate LPS response in cells that do not express cell surface CD14.

References:

1. Wright, S.D. *et al.* (1990) *Science* **249**:1431.
2. Pugin, J. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90**:2744.
3. Beutler, B. (2000) *Current Opinion in Immunology* **12**:20.
4. Stetter, F. (2000) *Chem. Immunol.* **74**:25.