

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Collagen IV $\alpha 1$ in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant mouse COL13A1, recombinant human (rh) COL25A1 and rhCOL23A1 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Collagen IV $\alpha 1$ Ser1441-Thr1669 Accession # P02462
Formulation	Lyophilized from a 0.2 μ m 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 μ m filtered solution in PBS.

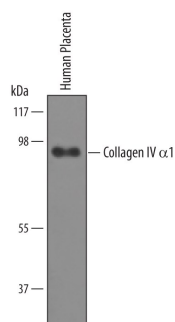
APPLICATIONS

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	1 μ g/mL	See Below
Immunohistochemistry	5-25 μ g/mL	Immersion fixed paraffin-embedded sections of Human Kidney

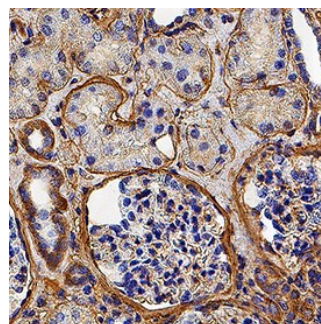
DATA

Western Blot



Detection of Human Collagen IV $\alpha 1$ by Western Blot. Western blot shows lysates of human placenta tissue. PVDF Membrane was probed with 1 μ g/mL of Human Collagen IV $\alpha 1$ Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6308) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Collagen IV $\alpha 1$ at approximately 95 kDa (as indicated) ref 1. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Immunohistochemistry



Detection of Collagen IV $\alpha 1$ in Human Kidney. Collagen IV $\alpha 1$ was detected in immersion fixed paraffin-embedded sections of Human Kidney using Sheep Anti-Human Collagen IV $\alpha 1$ Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6308) at 5 μ g/mL for 1 hour at room temperature followed by incubation with the Anti-Sheep IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC006). 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 connective tissue. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
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

COL4A1 (collagen 4- α 1) is a calculated 185 kDa (ref. 1) member of the type IV collagen family. It is a secreted glycoprotein that is expressed by multiple cell types, including fibroblasts, keratinocytes, and endothelial cells. Two COL4A1 molecules interact with a 170 kDa α 2 chain to form a collagen IV triple helix. This helix further interacts with other helices to generate covalent oligomers that form a scaffold in the basement membrane. Mature human COL4A1 is 1642 amino acids (aa) in length (SwissProt #:P02462). It has an N-terminal "7S" proregion (aa 28-172), a central collagenous domain that contains multiple Gly-based repeats (aa 173-1440), and a C-terminal domain that is proteolytically cleaved to generate a 25-28 kDa NC1 globular segment that has potent antiangiogenic activity (aa 1441-1669). Multiple splice forms exist. One shows a deletion of aa 499-849, a second shows a seven aa substitution for aa 513-1669, and a third shows a seven aa substitution for aa 958-1669. Over aa 1441-1669, human COL4A1 shares 97% aa sequence identity to mouse COL4A1. AF6308 detects a 95 kDa band, endogenous COL4A1 by Western blot in human placenta tissue sample, which is consistent with the current literature (ref. 1)

References:

1. Protein Science (2006), **15**:2805.