**Product datasheet**

**Anti-Collagen III antibody ab7778**

**Product name**  
Anti-Collagen III antibody

**Description**  
Rabbit polyclonal to Collagen III

**Host species**  
Rabbit

**Specificity**  
This type specific collagen antibody only recognizes 3D epitopes. Negligible cross-reactivity with Type I, II, IV, V or VI collagens. Non-specific cross reaction of anti-collagen antibodies with other human serum proteins or non-collagen extracellular matrix proteins is negligible.

**Tested applications**  
Suitable for: IHC-P, ICC/IF, ICC, ELISA, IHC-Fr, IP, WB

**Species reactivity**  
Reacts with: Mouse, Rat, Cow, Human

**Predicted to work with:** Mammals

**Immunogen**  
Full length native protein (purified) corresponding to Collagen III aa 1-1466.  
Database link: P02461

**Positive control**  
Natural Cow Collagen III protein (ab7528) can be used as a positive control in WB. Human skin. Human testicle tissue.

**General notes**  
For more protocol tips, please see: https://www.abcam.com/protocols/collagen

At least 11 genetically distinct gene products are collectively referred to as ‘collagen types’ or other proteins and proteoglycans of the extracellular matrix. In humans, collagens are composed of about 20 unique protein chains which undergo various types of post-translational modifications and are ultimately assembled into a triple helix. This results in great diversity between collagen types. Collagens are highly conserved throughout evolution and are characterized by an uninterrupted "Glycine-X-Y" triplet repeat that is a necessary part of the triple helical structure. For these reasons it is often extremely difficult to generate antibodies with specificities to collagens. The development of type specific antibodies is dependent on NON-DENATURED three-dimensional epitopes. This preparation results in a native conformation of the protein.

Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).

See other anti-rabbit secondary antibodies that can be used with this antibody.

**Properties**

**Form**  
Liquid

**Storage instructions**  
term. Avoid freeze / thaw cycle.

Storage buffer
- pH: 7.20
- Preservative: 0.01% Sodium azide
- Constituents: 0.8766% Sodium chloride, 0.424% Potassium phosphate

Purity
- Immunogen affinity purified

Purification notes
- Immunooaffinity chromatography using immobilized antigens followed by extensive cross-adsorption against other collagens, human serum proteins and non-collagen extracellular matrix proteins to remove any unwanted specificities.

Clonality
- Polyclonal

Isotype
- IgG

Applications

Our Abpromise guarantee covers the use of ab7778 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>IHC-P</td>
<td>🟢🟢🟢🟢 🟡</td>
<td>Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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<tr>
<td>ICC/IF</td>
<td>🟢🟢🟢🟢 🟡</td>
<td>Use at an assay dependent concentration. PubMed: 18385800</td>
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<tr>
<td>IF</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 28135282</td>
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<tr>
<td>ICC</td>
<td></td>
<td>1/100. PubMed: 19036760</td>
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<tr>
<td>ELISA</td>
<td></td>
<td>1/1000 - 1/8000. This product was assayed against 1.0 ug of Collagen III in a direct ELISA using Peroxidase conjugated Goat anti-Rabbit and ABTS (2,2'-azino-bis-[3-ethylbenzthiazoline-6-sulfonic acid]) as a substrate for 30 minutes at room temperature. This product can also be used with Biotin Conjugated Anti-Collagen III (ab6580) in a sandwich ELISA.</td>
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<tr>
<td>IHC-Fr</td>
<td>🟢🟢🟢🟢 🟡</td>
<td>1/50 - 1/200.</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>1/5000 - 1/10000.</td>
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</table>

Target

Function
- Collagen type III occurs in most soft connective tissues along with type I collagen.

Involvement in disease
- Defects in COL3A1 are a cause of Ehlers-Danlos syndrome type 3 (EDS3) [MIM:130020]; also known as benign hypermobility syndrome. EDS is a connective tissue disorder characterized by hyperextensible skin, atrophic cutaneous scars due to tissue fragility and joint hyperlaxity. EDS3 is a form of Ehlers-Danlos syndrome characterized by marked joint hyperextensibility without skeletal deformity.
- Defects in COL3A1 are the cause of Ehlers-Danlos syndrome type 4 (EDS4) [MIM:130050]. EDS is a connective tissue disorder characterized by hyperextensible skin, atrophic cutaneous scars due to tissue fragility and joint hyperlaxity. EDS4 is the most severe form of the disease. It is...
characterized by the joint and dermal manifestations as in other forms of the syndrome, characteristic facial features (acrogeria) in most patients, and by proneness to spontaneous rupture of bowel and large arteries. The vascular complications may affect all anatomical areas. Defects in COL3A1 are a cause of susceptibility to aortic aneurysm abdominal (AAA) [MIM:100070]. AAA is a common multifactorial disorder characterized by permanent dilation of the abdominal aorta, usually due to degenerative changes in the aortic wall. Histologically, AAA is characterized by signs of chronic inflammation, destructive remodeling of the extracellular matrix, and depletion of vascular smooth muscle cells.

Sequence similarities
Belongs to the fibrillar collagen family.
Contains 1 fibrillar collagen NC1 domain.
Contains 1 VWFC domain.

Post-translational modifications
Proline residues at the third position of the tripeptide repeating unit (G-X-Y) are hydroxylated in some or all of the chains.
O-linked glycan consists of a Glc-Gal disaccharide bound to the oxygen atom of a post-translationally added hydroxyl group.

Cellular localization
Secreted > extracellular space > extracellular matrix.

Images

Analysis of MSCs (mesenchymal stromal cells)
Collagen I, III, and IV complexes stained after 72 hours of culture on glass slides. DAPI stained nuclei are shown in gray. Merged images shown are representative of 3–4 slides per test condition.
Collagen III is detected using ab7778.
(From Figure 3H of Han et al).

A2AR activity affected UUO (unilateral ureteral obstruction)-induced deposition of collagen III
(A) Representative immunohistochemistry of Collagen III (Col III) from the A2AR KO and WT mice, at day 3, 7 and 14 post-UUO or Sham, following treatment of CGS21680 (CGS) or vehicle (Veh). Scale bar =50 μm, 400×.
Collagen III in rat kidney tissue is detected ab7778 at 1/400 dilution.
Pressure cooker mediated antigen retrieval was performed.
(From Figure 3 of Xiao et al)
ab7778 staining Collagen III in human testicle tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).

Tissue was fixed with paraformaldehyde and a heat mediated antigen retrieval step was performed using TE buffer pH 9.0. Samples were then incubated with ab7778 at a 1/200 dilution for 30 minutes at 20°C. The secondary used was an undiluted, HRP-conjugated goat anti-rabbit polyclonal.

Anti-Collagen III antibody (ab7778) at 1/1000 dilution + Human collagen III at 0.1 µg

**Predicted band size:** 138 kDa

Western Blot produced under denaturing and reducing conditions.

ab7778 staining Collagen III in Human saphenous vein-derived myofibroblasts by Immunocytochemistry/ Immunofluorescence.

Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton-X100. Prior to staining, cells were washed and blocked in a 2% bovine serum albumin, 0.1% saponin (w/v) solution in PBS, and subsequently incubated for 1 hour with the primary antibody in PBS. Nuclei are stained with Hoechst.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Collagen III antibody (ab7778)

**ab7778 at 1:400 (45 min RT)** showed strong staining in FFPE sections of human skin (left, dermis) with moderate to strong red staining and testis (right) where strong staining was observed within connective tissue between seminiferous tubules. The antibody showed strong extracellular staining within connective tissues across many organs with minimal background staining. Slides were steamed in 0.01 M sodium citrate buffer, pH 6.0 (ab64214) at 99-100°C - 20 minutes for antigen retrieval.

Immunohistochemistry of ab7778. Tissue: right lobe of the liver section. A: Central Vein (CV) fibrosis, B: Non-fibrotic CV, C: Perisinusodial fibrosis, D: Non-fibrotic area, E: Protat tract fibrosis, F: Septal fibrosis (arrow). Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: Anti-collagen type I at 1:500 for 4°C for 24 hr. Secondary antibody: Peroxidase biotin-streptavidin rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: Anti-collagen type III is intra and extracellular. Staining: 3,3’-diaminobenzidine tetrahydrochloride was used as the chromogen. Nuclei were counterstained purple with hematoxylin.

Ab53088 staining Human skin (ab30166). Staining is localised to the extracellular matrix.

Left panel: with primary antibody at 1 ug/ml. Right panel: isotype control.

Sections were stained using an automated system DAKO Autostainer Plus, at room temperature. Sections were rehydrated and antigen retrieved with the DAKO 3-in-1 antigen retrieval buffer citrate pH 6.0 (ab64214) in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight
incubation), and amplification may be required.

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