

Collagen I

Western Blot

<https://www.abcam.com/protocols/general-western-blot-protocol>

Target Overview in WB Application

Collagen I is the major protein component of the extracellular matrix in bone, skin and tendon, mainly secreted by osteoblasts, dermal fibroblasts and tenocytes. **Proper collagen isolation** method is critical to aiding WB success.

Here are a few tips to help ensure the best results in WB:

<https://www.abcam.com/protocols/collagen>

Electrophoresis	<ul style="list-style-type: none"> • Use a 6% acrylamide gel for collagen I.
Transferring	<ul style="list-style-type: none"> • It is preferred to add SDS to a final concentration of 0.1% in the transfer buffer for large proteins. • Wash PVDF membrane to remove methanol completely. • To determine if the transfer is successful by visualization of proteins in membranes using Ponceau S.

You should pay attention to these notes to maximize the signal:

- ✓ **Acid or enzyme treatment with pepsin** is a better method to isolate collagen.
- ✓ **Continuous refrigeration** throughout collagen extraction is important to avoid degradation and denaturation.
- ✓ Take care with **pH, temperature, and concentration** to avoid collagen polymerization:
 - Collagen is soluble in acidic condition so an **acidic pH** is critical for collagen stability and solubility.
 - At a basic pH, collagen will polymerize and form a hydrogel.
 - Too high concentrations of collagens loaded can also lead to polymerization.
 - As collagens are more unstable than other proteins, WB should be **performed at <10°C**.
- ✓ **ab138492** has not been experimentally confirmed in cell lysates in western blot.
- ✓ If you are using **ab34710**, the collagen antibody which is developed using non-denatured 3D epitopes, you must be careful **not to denature the collagen protein** during your experiment.
- ✓ We recommend using **native protein standards** as positive and negative controls for ab34710.

Protein Function	<p>Collagen I is a member of group I collagen (fibrillar forming collagen). It's a protein that strengthens and supports many tissues in the body, including cartilage, bone, tendon, skin and the white part of the eye (sclera). Collagen I triple helix comprises of two alpha1 chains and one alpha2 chain. COL1A1/A2 could be useful for detecting melanoma, lung, liver, glioma, skin, stomach, and other cancers.</p> <p style="text-align: right;">SwissProt: P02452</p>
Expression	<p>Forms the fibrils of tendon, ligaments and bones.</p> <p>In bones the fibrils are mineralized with calcium hydroxyapatite.</p>
Location	<p>Secreted > extracellular space > extracellular matrix.</p>
Isoforms	<p>- Isoform 1: 139 kDa (predicted)</p>
Modifications	<p>Hydroxylation/O-glycosylation</p> <p>The observed band size of Collagen I may not be the same as predicted MWs in WB due to these modifications.</p>
Positive Controls	<p>WB: Human stomach, skin and adrenal gland tissue lysates.</p>