

## Product datasheet

# Total Collagen Assay Kit (Perchlorate-Free) ab222942

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### Overview

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<b>Product name</b>	Total Collagen Assay Kit (Perchlorate-Free)
<b>Detection method</b>	Colorimetric
<b>Sample type</b>	Urine, Serum, Tissue Homogenate
<b>Assay type</b>	Quantitative
<b>Range</b>	0.5 µg - 10 µg
<b>Assay time</b>	3h 00m
<b>Species reactivity</b>	<b>Reacts with:</b> Mammals, Other species
<b>Product overview</b>	Total Collagen Assay Kit (Perchlorate-Free) (ab222942) provides a quick and convenient method to detect collagen in tissue lysates as well as biological fluids such as urine or serum.

The collagen assay protocol is based on the Alkaline hydrolysis of samples to yield free Hydroxyproline. The released hydroxyproline is oxidized to form a reaction intermediate, which further in reaction forms brightly-colored chromophore that can be easily detected at OD 560 nm.

The assay employs a proprietary developer solution to accurately measure collagen in hydrolysates without the use of hazardous perchloric acid, removing the need for any special handling and waste-disposal protocols. The assay can detect as low as of 0.5 µg collagen/well.

Collagen assay protocol summary:

- hydrolyze samples and standard with concentrated NaOH for 1 hr at 120°C
- cool on ice and neutralize with concentrated HCL
- centrifuge at 10,000g for 5 min to pellet debris
- add samples and standards to wells
- evaporate wells to dryness at 65°C
- add oxidation mix and incubate for 20 min
- add developer and incubate for 5 min
- add DMAB concentrate and incubate for 45 min at 65°C
- analyze absorbance at 560 nm with a microplate reader

**Notes** This product is manufactured by BioVision, an Abcam company and was previously called K406 Total Collagen Assay Kit (Perchlorate-Free). K406-100 is the same size as the 100 test size of ab222942.

Collagen is the most abundant insoluble protein found in the extracellular matrix and connective

tissues. It can be found in skin, tendons, bone, cartilage, muscle, vitreous humor and ligaments, among other tissues. There are more than sixteen - well characterized types of collagens, but types I, II and III collagen comprise more than 80% content in mammals.

**Platform**

Microplate reader

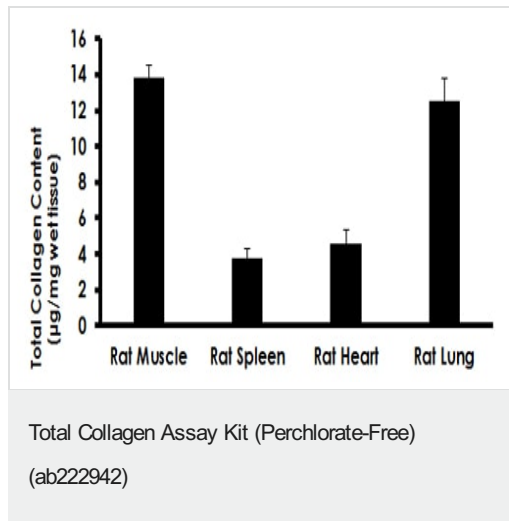
**Properties**

**Storage instructions**

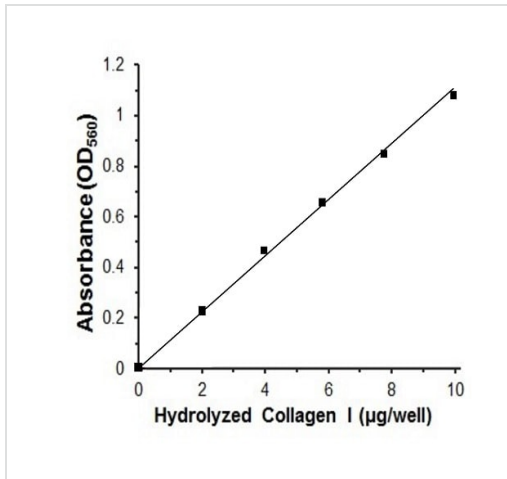
Please refer to protocols.

Components	100 tests
Chloramine T Concentrate	1 x 600µl
Collagen I Standard	1 x 200µl
Developer	1 x 5ml
DMAB Concentrate	1 x 5ml
Oxidation Buffer	1 x 10ml
Plate sealer	1 unit

**Images**

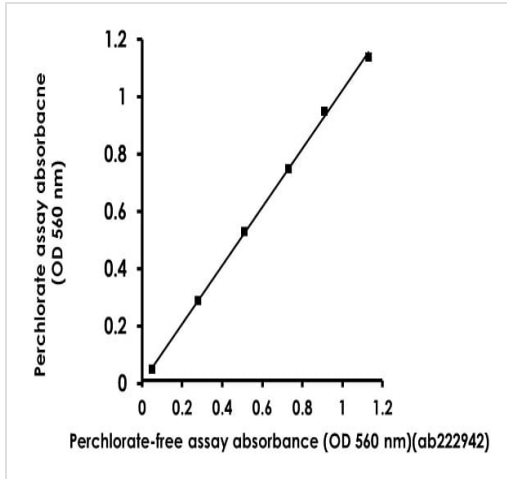


Estimation of total collagen content in rat tissues. Rat leg muscle, spleen, heart and lung samples were prepared following the sample preparation procedure described in the protocol (homogenized in ddH<sub>2</sub>O, hydrolyzed with 10 N NaOH for 1 hour at 120°C and neutralized with 10 N HCl). For each sample, 10 µL of the final neutralized hydrolysate was assayed. Total Collagen levels (calculated as µg collagen/mg wet tissue) for the samples were: 13.80 ± 0.73 ug/mg for muscle, 3.77 ± 0.54 ug/mg for spleen, 4.55 ± 0.81 ug/mg for heart and 12.51 ± 1.27 ug/mg for lung. Data are mean ± SEM of 3-4 replicates.



Typical hydrolyzed Collagen I standard calibration curve.

Total Collagen Assay Kit (Perchlorate-Free)  
(ab222942)



Correlation between collagen standard curve absorbance values obtained using a classical perchlorate-based assay kit and perchlorate-free Total Collagen Assay Kit (Colorimetric) (ab222942).

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