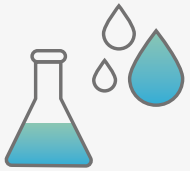
PROMED BIOSCIENCE

Recommendations for reconsitution & use

If possible, we recommend to carefully and aseptically remove the lyophilizate from the packed vial, weight it and perform the reconstitutions in a separate container.

It is also advisable to use a transparent container for your reconstitution, in order to be able to assess the clarity of the solution.



More questions about how to use the PRO collagen sample?

Check the applications listed in the product information brochure, which is supplied with the vial packaged. Alternatively, we are one **email** message away!

You can contact us through our **website** for application protocols, videos and other specific enquiries you may have!

Which solvent to use?

Use a recently-prepared (less than 6 months) solution of weak Acetic or Hydrochloric acid.

Measure the pH and ensure it is within the range of 1.8 – 2.9. It is recommended to use 0.1 M Acetic acid or 0.01 M Hydrochloric acid.

Extra precautions during initial reconstitution

Reconstitute initial stock solution at a concentration of 0.5 – 2.0 mg/mL by gradual and slow addition of acid; do not add the whole diluent amount at once. Rather, add the appropriate volume of diluent in 3-4 + portions.

For example, if 3 mL is to be added to the lyophilizate, add 1 mL at a time and mix well. Based on trials and past experience, the lyophilizate is very sensitive to pH changes and Ac Acid additions.

Therefore, at the beginning of reconstitutions, acid must be added gradually, with pipetting and manual mixing, to ensure that the solvent is distributed thoroughly within the lyophilizate.

EXTRA HINT
Add 1 mL of diluent, distribute well through the lyophilizate(by pipetting and manual stirring using the pipette)
& incubate for at least 10 min before adding additional amount).

EXTRA HINT
If the sample does not dissolve completely after the aforementioned applications, warming up to 37*C may help.

Mixing technique matters!

DO not use vortexing – this will disrupt the collagen form and crosslinking. In order to dissolve the lyophilizate, it is advised to use gentle mixing techniques, including pipetting, manual swirling using a pipette tip or slight agitation/rotation of the tube.

How do I know that the reconstitution is complete?

Reconstitution must result in a homogenized mixture. Swirling mixture against the light can indicate if there is any undissolved material. Generally, atelocollagen solutions are less clear (off-white appearance) compared to telocollagen solutions, which have a clear appearance.

I am observing bubbles during the reconstitution – is this normal?

Bubbles may form, especially in the reconstituted telocollagen solution, which is normal. Bubbles usually dissolve over time when the sample is left to sit.

How long can the incubation last during reconstitutions?

It is normal to leave sample in diluent from 10 mins to hours and overnight, if required for complete reconstitution.

How to store reconstituted sample?

Store reconstituted sample at 2 – 8°C.

What if further dilution is required?

As mentioned above, we recommend initial reconstitution at around 2.0 mg/mL and no less than 0.5 mg/mL.

If a further dilution is required by your individual protocol, add additional diluent gradually, following the initial reconstitution. With each addition of solvent, perform adequate mixing and allow the sample to incubate at RT for some time (at least 10 mins). Ensure that the solution is homogeneous before proceeding to add more diluent.

How to sterilize the reconstituted sample?

We recommend sterilizing the reconstituted sample in 10% chloroform. Briefly, add collagen solution in a glass container, then carefully and slowly pipette 10% v/v chloroform in the bottom and at the middle of the container. Two layers will be formed. Leave closed container overnight at RT. Next day, collect the top layer carefully (sterilized collagen solution), without touching the bottom layer (chloroform). The collected collagen is sterilized and ready for tissue culture.

Regarding other commonly used sterilization techniques:

- We do not advise using filter-sterilization as significant amount of collagen may be lost. Clogging of the filter unit may also occur.
- UV sterilization may interfere with the natural collagen crosslinking.