We would be happy to work with you to optimise this procedure for your applications. If you would like any advice on the method and use of collagen, please contact the ProColl team info@procoll.co.uk

Research Grade Collagen Gelling Protocol 2 - *Cross linker free*

When using acid soluble collagen, pH neutralisation in buffered conditions causes the collagen molecules to become insoluble and aggregate to form a gel when the pH is neutral. This forms a weak gel and chemical cross-linking is recommended to produce a stiffer gel that is better suited to the cell growth application. When a higher concentration is used, such as our recommended starting concentration of 6 mg/mL, a robust gel can be formed without the use of a crosslinking agent. There are a number of chemical cross-linking methods that can be used to strengthen collagen gels which include glutaraldehyde, genipin, EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide, dialdehyde starch and chitosan. Avoiding the use of crosslinking within your workflow can allow the removal of variables and simplification of the hydrogel environment. This procedure can be carried out at room temperature or incubated to match cell solutions' optimal conditions.

Prepare the collagen gel as follows:

- Add 1 PBS tablet to 20 mL deionised water to produce a 10X PBS solution (or equivalent amount to normally produce 1X PBS in 200 mL). Alternatively, media such as HBSS can be used in place of deionised water.
- Store the PBS solution at 4°C until ready to use.
- Add 1 mL PBS solution to 9mL of collagen solution at 6mg/mL or higher. This will produce a 10 mL solution of 5.4 mg/mL in 1X PBS.
- Raise the pH of the collagen solution to between 6.0 and 8.5 using 1% (0.25M) and 10% (2.5M) NaOH solutions. (Do not overshoot, too high a pH will cause irreversible denaturation to the collagen).
- Once desired pH is reached, place at either 4°C, room temperature, 25°C or 37°C.
- If creating a cell-loaded hydrogel solution, combine with cellular mixture at this point, ensuring collagen concentration remains above 3 mg/mL.
- Gelling at pH 7.0 – 7.8 occurs completely in <10 minutes at RT in 3.0 mg/mL or higher solutions.