POLBIONICA
We print organs. We do science not fiction!

INSTRUCTIONS FOR THE PREPARATION OF HYDROGEL dECM-PAN Printiss®

(dECM-PAN - decellularised extracellular matrix from porcine pancreas)

The instructions are intended as auxiliary recommendations.
The user can make appropriate changes, taking into account his hardware resources.

This manual has been prepared and published by POLBIONICA sp. z o.o.

PRELIMINARY INFORMATION:

Instructions for the preparation of a dECM-PAN hydrogel with a final concentration of 1.0% (w/v) in a 12.5 ml volume.

***Note:* due to the neutralisation step, the initial hydrogel concentration is 1.25% (w/v)**

MATERIALS:

Materials supplied by the manufacturer:

- dECM powder (sterile) - 1 pc. (125.0 mg)
- pepsin (powder, sterile) - 1 pc. (10.0 mg)
- 0.1 M NaOH – 1 pc. (10.0 ml)
- 0.1 M HCl – 1 pc. (10.0 ml)
- PBSx10 - 1 pc. (10.0 ml)

KEY TIPS:

1. You are advised to perform all activities using a laminar flow cabinet to maintain sterility.
2. The dECM-PAN powder and pepsin supplied are sterile, but we recommend filtration of the other reagents and using sterile instruments and consumables to maintain complete sterility.
3. We recommend that all reagents are filtered through a 0.22µm syringe filter before use (except the final dECM-PAN solution).
4. Before proceeding with the hydrogel preparation procedure, prepare 0.01 M HCl and PBSx1 solutions using the materials supplied by the manufacturer.
5. The recommended storage time to maintain the initial properties of the hydrogel is one month at 4° C.

PROCEDURE:

a. Digestion of dECM-PAN

1. Measure out 10.0 ml of sterile 0.01 M HCl solution and transfer it to a glass bottle with the stirring element inserted.
2. Weigh out 10.0 mg of pepsin and transfer it to a bottle with 0.01 M HCl.

   ***Note:* this gives a pepsin concentration = 1 mg/ml**
3. Stir the solution for 10-15 minutes at 500 rpm at room temperature until the enzyme fully dissolves.
4. Add 125.0 mg of dECM-PAN powder to the prepared solution and then place the suspension in a water bath (30°C) on a magnetic stirrer. Set the speed to 500-600 rpm.
5. Run the digestion process continuously for 72h maintaining stable conditions.

b. Hydrogel neutralisation

1. Perform the neutralisation process in a laminar flow cabinet.
2. To the hydrogel solution, add 1.111 ml of PBSx10, 0.389 ml of PBSx1 and 1.0 ml of 0.1 M NaOH in 5 portions (200 µl); after each alkali addition, the solution should be stirred.

   Note: *the amount of PBSx10 and 0.1 M NaOH added is closely related to the amount of acid used in the dECM--PAN digestion step; when preparing a different volume of hydrogel, the ratio of the above reagents to each other must be maintained; the amount of PBSx1 added depends on the expected final hydrogel concentration, in this case, the final hydrogel concentration is 1.0% (w/v); obtaining a lower hydrogel concentration requires the addition of a correspondingly larger amount of PBSx1*
3. The neutralisation process should be carried out until a stable pH of 7.2-7.4 is reached.
4. The neutralised hydrogel should be sealed and stored at 4° C until use.

   Note: *hydrogel stored at 4° C may gel, but it is reversible*
5. Before use, the hydrogel should be placed in a water bath at 30° C and allowed to liquefy fully.

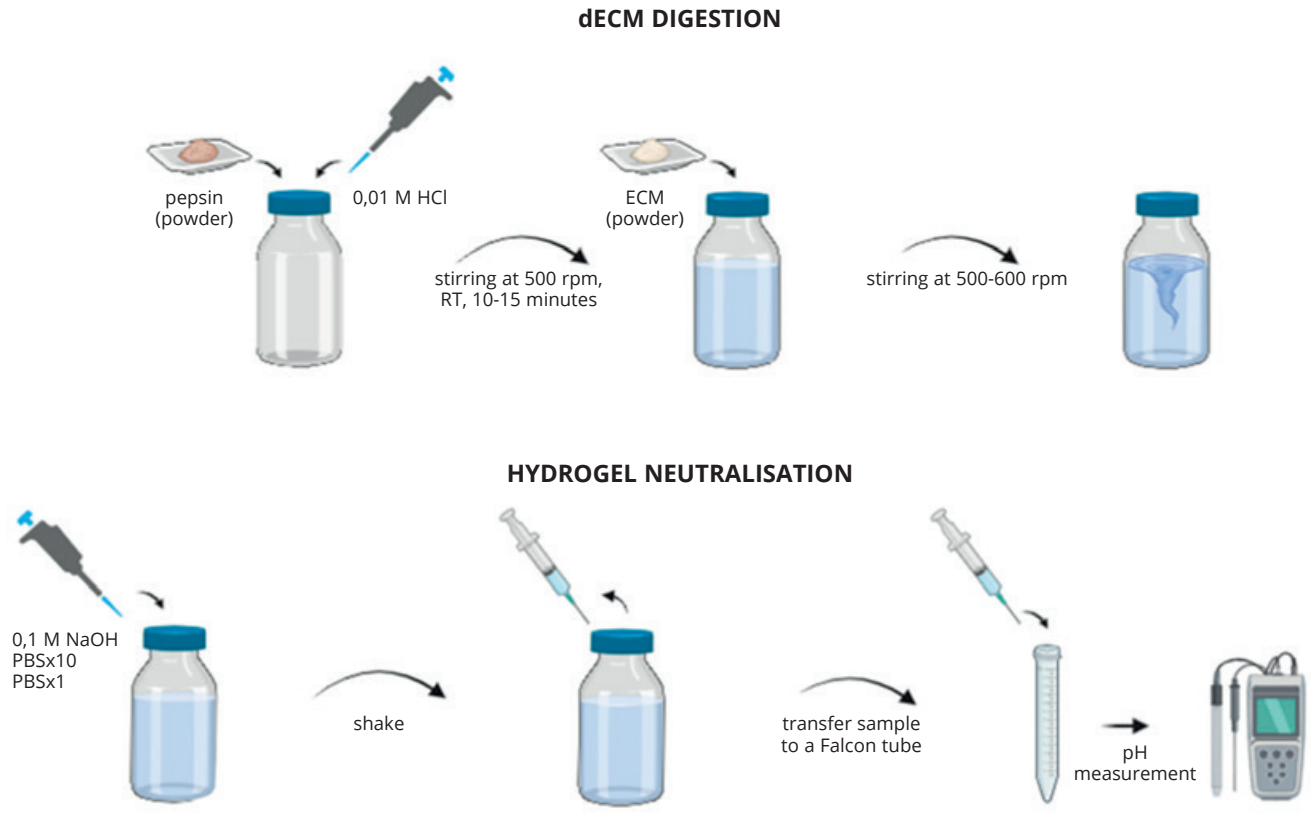

Figure1. *Illustration of hydrogel preparation*