

Präparation of epidermal sheets by ammonium thiocyanate

Buffers:

Sodium Phosphate Buffer:

1 M Na_2HPO_4 (Applichem A 2943.0500, water-free, $M=141,96$ g/mol)

1 M NaH_2PO_4 (Applichem A 1047.1000, monohydrate; $M=137,99$ g/mol)

Preparation of 0,1 M Sodium Phosphate Buffer at 25 °C (red book)

pH 6,8:

prepare 46,3 ml 1 M Na_2HPO_4 with 53,7 ml 1 M NaH_2PO_4

Preparation of 0,5 M ammonium thiocyanate in 0,1 M Sodium Phosphate Buffer pH 6,8

Ammonium thiocyanate A7149 (Sigma); $M= 76,12$ g/mol

100 ml > 3,81 g Ammonium-Thiocyanate ad 100 ml with 0,1 M Sodium Phosphate buffer, pH 6,8

50 ml > 1,91 g Ammonium-Thiocyanate ad 50 ml with 0,1 M Sodium Phosphate buffer, pH 6,8

Prepare fresh!

- Warm-up 0,5 M Ammonium-Thiocyanate in 0,1 M Sodium Phosphate Buffer, pH 6,8 to 37 °C
- Prepare full-thickness skin
- Spread skin by adding 0,5 M Ammonium-Thiocyanate solution
- Incubate 20 - 25 min (max) at 37 °C
- (Rinse in PBS)
- Take off epidermis use for subsequent applications
- (Rinse in PBS)
- (For RNA extraction RNase free Buffers are needed!)