

SOP: M020.1

Modified on 01/13/23 DRM

Preparation of Proskauer and Beck minimal media

Materials and Reagents:

1. Milli-Q water
2. pH meter
3. Magnetic stir bar
4. Magnetic stir plate
5. Potassium phosphate monobasic (Sigma cat#60218-500g)
6. L-Asparagine (Sigma cat#A4159-500g)
7. Magnesium sulfate hepta-hydrate (Macron cat# 6066-04)
8. Magnesium Citrate (Pfaltz+Bauer cat#M00235 100g)
9. Glycerol (Acros Organics cat#332030025)
10. (optional) Tween 80, 20% solution, sterile (G Biosciences cat#786-519)
11. Vacuum
12. 0.2 µm filter VacuCap
13. Bottle, 1L, 2x clean sterile bottle
14. Sodium hydroxide, 10 M

Protocol:

1. _____ Dissolve the following salts in 980mL on Milli-Q water into the 1L beaker in the order listed. Make sure reagent is completely dissolved before added the next, let solution stir approximately 5 minutes between additions.

	Volume	<u>1L</u>	<u>4L</u>
_____	MilliQ water	980mL	3920mL
_____	Potassium phosphate monobasic- KH_2PO_4	5.0g	20g
_____	L-Asparagine	5.0g	20g
_____	Magnesium sulfate hepta-hydrate- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.6g	2.4g
_____	Magnesium Citrate	2.5g	10g
_____	Glycerol	20mL	80mL
_____	Optional: add Tween 80 (20% solution) for a final concentration of 0.05%. Not for pellicle growth.	2.5mL	10mL

2. _____ Let the solution stir until clear. Add low heat from stir/hot plate if solution remains cloudy.

3. _____ Adjust pH to 7.4 with the addition of 10M sodium hydroxide. ~4mL ~16mL

4. _____ Pass media through a 0.2µm filter into a clean, sterile bottle. Aliquot to smaller volumes under sterile conditions.