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🌐 Buffer preparation for OnePot PURE cell-free system

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Protocol status: Working
We use this protocol and it's working

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Abstract

In this protocol we explain the procedure to create the Buffers used for Protein and Ribosome purification for the production of OnePot PURE cell-free system.

Materials

Material/Consumables:

- HEPES *(Sigma-Aldrich: H0887-100ML) pH=7.6 KOH*
- Magnesium acetate *(Sigma-Aldrich: M0631)*
- Magnesium chloride *(Sigma-Aldrich: 63020-1L)*
- Potassium chloride *(Sigma-Aldrich: P5405-1KG)*
- Ammonium chloride *(Sigma-Aldrich: 09718-250G)*
- Ammonium sulfate *(Sigma-Aldrich:A4418) pH=7.6 KOH*
- Sucrose *(Sigma-Aldrich: 84097)*
- β-Mercaptoethanol *(Sigma-Aldrich: M6250-100ML)*
- Imidasol *(Sigma-Aldrich: I2399) pH=7.6KOH*
- Glycerol *(Sigma-Aldrich: G7757-1L)*
- Distilled water

Equipment:

- Flow Bottle Top FIlter with 0.22μm aPES membrane
- Syringe
- Syringe Filter with 0.22μm PES membrane
- Beakers
- Glass Bottles for storage
- Magnetic stirrer

Safety warnings

> ❗ When handling β-Mercaptoethanol the researcher should work in a chemical hood and wear protective glasses.

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Add the materials needed the buffer you want to produce in a beaker. The final concentration of the components for the different buffers is presented in *Table 1* and *Table 2*

1.1

Table 1: Buffers for Protein Purification

Compound	Buffer A	Buffer B	Buffer HT	Stock buffer B
	mM	mM	mM	mM
HEPES	50	50	50	50
Ammonium chloride	1000			
Magnesium chloride	10	10	10	10
Potassium chloride		100	100	100
Imidasol		500		
Glycerol				60%
β-mercaptoethanol	7	7	7	7

> **Note**
>
> In Stock Buffer B, Glycerol should consists of 60% of the final volume

1.2   Table 2: Buffers for Ribosome Purification

Compound	Suspension buffer	Suspension buffer high salt	Buffer C	Buffer D	Cusion buffer	Ribosome buffer
	mM	*mM*	*mM*	*mM*	*mM*	*mM*
HEPES	10	10	20	20	20	20
Magnesium acetate	10	10	10	10	10	6
Potassium chloride	50	50				30
Ammonium chloride					30	
Ammonium sulfate		3000	1500			
Sucrose					30%	
β-mercaptoethanol	7	7	7	7	7	7

> **Note**
>
> In Cusion buffer, Sucrose should consists of 30% of the final volume

2   Add some distilled water and mix with a magnetic stirrer until all the solid components are dissolved

> **Note**
>
> Be careful so the volume at this point does not surpass the desired one, otherwise the components' concentration will be wrong on the final product.

3   Add as much distilled water is needed to reach the final volume of the buffers

4   Filter the solution.

─────────────── STEP CASE ───────────────

Filtering of Stock buffer B   2 steps

Use a syringe filter with 0.22µm PES membrane to filter the buffer before storing it.
Due to the viscosity of glycerol, it might be hard for the solution to be filtered but eventually all the solution will pass through.

5   The buffer must be stored at 4°C

6   Because β-mercaptoethanol has a short half-life we need to add it again to the buffer every time before we perform an experiment.