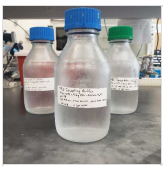

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Making MES Buffers for Protein EDAC Particle Coupling

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1 Works for me dx.doi.org/10.17504/protocols.io.bmndk5a6

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SUBMIT TO PLOS ONE

ABSTRACT

This protocol describes how to make MES buffers for covalently coupling proteins to carboxylated polystyrene particles via EDAC. Adapted from Bangs Laboratories Inc. TechNote 205 Covalent Coupling.

Three buffers are made from 2-(*N*-morpholino)ethanesulfonic (MES) acid with varying pH. The three buffers are referred to as :

- Activation buffer (acidic for activating the carboxyl group on particles for EDAC reaction)
- Coupling buffer (alkaline for coupling protein to o-acylisourea on particle surface created by EDAC reaction)
- Storage buffer (for storing particles while preventing degradation and self aggregation)

ATTACHMENTS

bangs_laboratories_technote205_covalent_coupling.pdf

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PROTOCOL CITATION

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KEYWORDS

MES buffer, EDAC, particle conjugation, protein conjugation, carboxylated microparticles, carboxyl modified microparticles, polystyrene partices, carbodiimide

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GUIDELINES

When adjusting pH, be sure to mix well and check pH continuously or frequently (if using pH strips).

MATERIALS TEXT

MATERIALS

MES, free acid, monohydrate **Bio Basic Inc. Catalog #MB0341.SIZE.100g**

BSA **Sigma Aldrich Catalog ##A8806**

Hydrochloric Acid **Fisher Scientific Catalog #A144S**

Sodium hydroxide **Sigma-aldrich Catalog #306576**

Reagents:

- MES (2-(*N*-morpholino)ethanesulfonic) acid
- Deionized water (DW)
- Sodium hydroxide (NaOH) solution, [M]**1 Molarity (M)**
- Hydrochloric acid (HCl), [M]**1 Molarity (M)**
- Bovine serum albumin (BSA)

Materials:

- pH meter or strips

BEFORE STARTING

Ensure that pH meter is calibrated.

Make MES Buffer Bulk

1 Combine:

- **12.8 g** MES free acid.
- **600 mL** DW.
- Swirl until MES is dissolved.

2 Adjust to ~6 pH. If pH is low add NaOH, if pH is high add HCl.

> Typically the solution is about pH**3** . In this case, adding about **16 mL** of NaOH [M]**1 Molarity (M)** should get you close. If the desired activation buffer pH is lower than pH**6** , adjust at this step to that pH.

Separate and Adjust

3 Separate the bulk buffer into the three buffers by transferring one third of the bulk to each of three glass bottles (~ **200 mL** each).

4 Adjust pH to the desired level for each buffer. NaOH is used to raise pH, HCl is used to lower pH. Recommended ranges from Tech Note 205 are shown below.

- Activation buffer: pH**4.5** - pH**7.5**
- Coupling buffer: pH**7.2** - pH**8.5**

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- Storage buffer: pH **7.0**

To reach ~ pH **8** Coupling buffer, about **13 mL** [M] **1 Molarity (M)** NaOH should be added.

To reach ~ pH **7** Storage buffer, about **10 mL** [M] **1 Molarity (M)** NaOH should be added.

Finish Storage Buffer

5 Add bovine serum albumin (BSA) to make [M] **0.1 % w/v** to Storage buffer (not Activation or Coupling buffer).

- For **200 mL** , add **200 mg** BSA.

Swirl to dissolve BSA.

BSA coats the particle surface, preventing nonspecific interaction (e.g. hydrophobic), also reducing self aggregation. The concentration of BSA can be varied from [M] **0.05 % w/v** - [M] **0.1 % w/v** . Additionally, surfactants may be used (e.g. Tween-80). Other blocking agents may be used in place of BSA.

Store Buffers

6 Store all buffers covered at **20 °C** .

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