

Protocol E: Preparation of pretreatment buffer

David Booth

Abstract

Citation: David Booth Protocol E: Preparation of pretreatment buffer. **protocols.io**

dx.doi.org/10.17504/protocols.io.h8xb9xn

Published: 22 Jun 2018

Protocol

Prepare pretreat buffer without reducing reagents

Step 1.

40 mM HEPES-KOH pH 7.5, 34 mM Lithium Citrate, and 15% (w/v) PEG 8000

Degas pretreatment buffer in a vacuum container

Step 2.

- Place open container of pretreatment buffer in an evacuated chamber
- This step is important to maintain the reducing potential of L-cysteine

Dissolve L-cysteine in degassed pretreatment buffer to a final concentration of 50 mM

Step 3.

- 60.6 mg of L-cysteine (Sigma 30089-25G) in 100 ml of pretreatment buffer

Sterile filter solution through 0.22 µm filter

Step 4.

- If using a vacuum driven filter, keep the vacuum on for 15 min to further degas the solution

Make 1 ml aliquots and store at -20°C

Step 5.