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Preparation of Sera-Mag SpeedBeads

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We use this protocol and it's working

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Disclaimer

The protocol outlined in this document was created as a part of the *Pooled, Growth-Based Assays for Protein Function Measurements* pipeline for Align to Innovate's Open Dataset Initiative. Align to Innovate is a non-profit research organization operating under open science principles with the goal of improving science research with programmable experiments. The Open Datasets Initiative is working to accelerate community-driven science with the use of automated labs to pioneer robust data collection methods and curated, high-fidelity, public biological datasets amenable to machine learning. This work was supported by Align to Innovate's Open Datasets Initiative which receives philanthropic funding in part from Griffin Catalyst.

Abstract

This protocol describes the preparation of Sera-mag SpeedBeads with PEG-salt buffer for downstream use in the **Automated Bar-Seq Library Preparation** protocol.

Materials

Instruments:

- Magnetic separation rack compatible with 2 mL microcentrifuge tubes
- Non-magnetic tube rack

Reagents:

- 15 mL of Sera-mag SpeedBeads Solution (MilliporeSigma 65152105050250)
- 15 mL of TE Buffer, Composition: 10 mM Tris-HCl (pH 8.0) and 0.1 mM EDTA (ThermoFisher 12090015)
- Polyethylene Glycol (PEG) 8000 (ThermoFisher 43443.36)
- Sodium Chloride (NaCl) 5M Solution (MilliporeSigma 59222C
- Tris-HCl 1M Solution, pH 8.0 (ThermoFisher J22638.K2)
- Ethylenediaminetetraacetic acid (EDTA) solution (Millipore Sigma E8008)
- Nuclease-free water (ThermoFisher 4387936)
- Tween 20 (MilliporeSigma P9416)

Consumables:

- 15 individual 2 mL microcentrifuge tubes (Celltreat 229446)
- 50 mL sterile conical tubes (ThermoFisher 339652)

Prepare SpeedBeeds in tubes

1 Vortex 15 mL Sera-mag SpeedBeads solution until beads are uniformly distributed.

2 Transfer 1 mL to each of the fifteen 2 mL microcentrifuge tubes.

3 Place all 15 of the tubes on the tube magnetic separation rack until beads are drawn to magnet.

4 Remove the supernatant from each tube.

Perform the first wash step

5 Add 1 mL TE buffer to each tube.

6 Remove the tubes from the magnetic stand and mix by vortexing

7 Return tubes to the magnetic separation rack and wait until beads are drawn to magnet.

8 Remove supernatant from each tube.

Perform the second wash step

9 Add 1 mL TE buffer to each tube.

10 Remove the tubes from the magnetic stand and mix by vortexing

11 Return tubes to the magnetic separation rack and wait until beads are drawn to magnet.

12 Remove supernatant from each tube.

Resuspend the beads

13 Add 1 mL TE to each tube and remove from magnet.

14 Fully resuspend by vortexing and set in a non-magnetic tube rack (not on magnetic stand).
- Note: Beads that have been washed and resuspended in TE can be stored in the refrigerator at this point and used for the following steps as needed.

STEP CASE

SeraPure beads/PEG/NaCl buffer preparation 11 steps

These steps detail how the washed and resuspended SpeedBeads can be used directly to make the beads/PEG/NaCl buffer preparation required downstream for the Automated Bar-Seq Library Preparation Protocol.

SpeedBeads magnetic beads/PEG/NaCl buffer preparation

15 Add 9 g PEG-8000 to a 50 mL sterile conical tube.

16 Add 10 mL 5 mol/L NaCl to the conical tube.

17 Add 500 µL 1 mol/L Tris-HCl, pH8 to the conical tube.

18 Add 500 µL 0.5 mol/L EDTA to the conical tube.

19 Fill the conical tube to ~45 mL using sterile nuclease free water.

20 Mix the conical tube by vortexing every 1-2 minutes until PEG goes into solution.
- When not vortexing, the tube can be placed on a heated shaker (e.g., Eppendorf ThermoMixer) at 50°C, 600 rpm, to reduce time required to dissolve the PEG
- When finished, the solution should be clear.

21 Add 27.5 µL Tween 20 to the conical tube and mix gently by inverting 4-6 times.

22 Mix two 1 mL aliquots of the SpeedBeads + TE solution until the solution is a uniform brown color, then transfer the solutions from both tubes to the 50 mL conical tube.

23 Fill the conical tube to the 50 mL mark with sterile nuclease free water

24 Gently mix until the solution is a uniformed brown color.

25 Wrap in the conical tube in foil (or place in dark container) and store at 4 °C until use.