

Cell staining buffer (CSB)

1. Prepare the following materials:

Item	Order number
PES 0.2 um filter	Fisherbrand FB12566510
Disposable Polystyrene Sterile Bottles 1L	Corning™ 430518
10X PBS pH 7.2	Rockland Immunochemicals MB0084000
30% BSA	Sigma Aldrich A728450ML
[Optional] 20% NaN ₃ (w/v) (200X)	Prepared in-house
Type 1 water	Prepared in-house

2. Prepare 20% NaN₃ solution by dissolving 10g of Sodium Azide in 50mL of Type 1 water. Filter through a 0.2um syringe filter into a 50-mL tube. Label and update Benchling inventory.
3. Refer to the reference table below for exact amounts of each chemical needed for 1L or 2L of cell staining buffer.

	Quantity for 1L	Quantity for 2L	Quantity for 4L
10X PBS	100 mL	200 mL	400 mL
30% BSA	16.7 mL	33.3 mL	66.6 mL
[Optional] 20% NaN ₃	5 mL	10 mL	20 mL
Type 1 water	Fill to 1000 mL	Fill to 2000 mL	Fill to 4000 mL

4. Mix liquids in a graduated cylinder or bottle with a magnetic stir bar.
5. Filter the solution through a PES 0.2um filter into a clean bottle(s).
Note: filtration here is to remove particles from the solution. Do not need to be handled inside BSC.
6. Label solution with solution name, operator initial, preparation date, expiration date (6 months), and “filtered” on the bottle.
Note: sodium azide is the active ingredient to inhibit microbial growth. If omitted, inspect every time before use to ensure no contamination.
7. Store the solution at 4°C.