

## Buffers for Human Liver and Tumor Immune Cell Isolation

few days before  
isolation

### 1. 25% BSA Aliquots (1 aliquot = 2 ml with 500 mg BSA total)

- dissolve 25 g BSA in 100 ml HBSS using a stirring fish
- filter through vacuum sterile filter under the hood
- aliquot in 2 ml Eppendorf tubes and store at -20 °C

### 2. Blocking Buffer

- transfer 1 gram of BSA to a 50 ml falcon
- add 1 ml of each type of sera (human, mouse, rabbit, rat)
- add PBS ad 50 ml
- mix until dissolved
- aliquot and store at -20 °C

### 3. GBSS

[mg]	GBSS
Glucose	991
KCl	370
Na <sub>2</sub> HPO <sub>4</sub> *2H <sub>2</sub> O	75
NaHCO <sub>3</sub>	227
CaCl <sub>2</sub> *2H <sub>2</sub> O	225
KH <sub>2</sub> PO <sub>4</sub>	30
MgCl <sub>2</sub> *6H <sub>2</sub> O	210
MgSO <sub>4</sub> *7H <sub>2</sub> O	70
dH <sub>2</sub> O to (ml)	1000

- combine reagents and 900 ml dH<sub>2</sub>O, mix by using a stirring fish
- when reagents are dissolved, set pH to 7.4
- add dH<sub>2</sub>O to a final volume of 1000 ml
- sterile filtrate through a vacuum sterile filter under the hood
- keep at 4 °C

1 day before  
isolation

### 4. Stop Digest Buffer

- add 2 ml 25% BSA and 2 ml 0,5 M EDTA a 500 ml HBSS bottle
- close the top and mix well by shaking
- keep at 4 °C

### 5. HBSS + 0,1% BSA

- add 2 ml 25% BSA to a 500 ml HBSS bottle
- close the top and mix well by shaking
- keep at 4 °C

### 6. FACS Buffer

- add 2 ml 0,5 M EDTA to a 500 ml bottle of PBS
- close the top and mix well by shaking
- keep at 4°C

the day of  
isolation

### 7. Nycodenz stock solution (1 sample)

- measure 4 g of Nycodenz and transfer to a 50 ml Falcon
- add 14 ml of GBSS to the Falcon
- close the top tightly and vortex until half of the Nycodenz has dissolved
- let the falcon rest on the side on the countertop for 5 min
- vortex again until dissolved completely
- keep on ice

**8. Fixation Buffer (2% PFA in PBS)**

- Dilute 4% PFA and PBS 1:1
- Keep at 4 °C

**9. 1X Lysis Buffer**

- Dilute 10X Lysis Buffer 1:10 in dH<sub>2</sub>O (e.g. 1 ml of Buffer with 9 ml of dH<sub>2</sub>O)
- Keep on ice