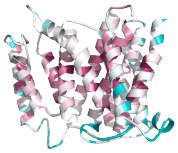
PROVOST & WALLERT RESEARCH

Investigating the Biochemistry &
Cellular Physiology of NHE1
EST. 1998

PSN, KR/A and E266I cell maintenance

Protocol



PS120 cells stably expressing *wild-type NHE1 (PSN),* mutated NHE1 with *impaired ERM binding (KR/A)* or *proton transport defective NHE1 (E266I)* cells are maintained in DMEM with high glucose, 10% heat inactivated fetal calf serum containing 0.4 mg/ml G418 (Calbiochem San Diego CA) and were kind gifts from Diane Barber University of California San Francisco CA.

PS120 are NOT resistant, do not culture them with G418!

Culturing Instructions: Diane recommended doing an acid load once after you have them (PSN and KR/A only) up. The purpose is to kill any of the cells that don't have a functional exchanger by 'loading' with acid and forcing the cells to extrude hydrogen (so you could use an extra dish of PS120s as controls, for example, since they should all die), so it's just to ensure you have a uniform population of cells. I'm almost certain they would have been acid-loaded once already, so most likely almost none will die off- but it's more of a housekeeping thing.

It's safest to pass into at least two 10cm dishes, and acid-load one while maintaining the other as is, in case of contamination or something. After they're fully recovered, I'd freeze and otherwise use/maintain normally. Let me know if you have any other questions

Acid Load Instructions:
Maintain cells in flask or dish and perform acid loading when cells are 50-80% confluent.

1. Wash cells twice with Hepes buffer
2. Add NH4CL buffer, tighten cap and incubate at 37°C in the absence of CO_2 for 60 min.
3. Wash cells twice with Hepes buffer
4. Add Hepes buffer, tighten cap and incubate at 37°C in the absence for CO_2 for 90 min.
5. Wash cells twice with Hepes buffer, add complete media and return to CO_2 incubator
6. Let the cells recover for 24 hrs or more before trypsinizing

Buffers

Hepes Buffer pH7.4	Final Conc	Stock Conc	For 500 mls
NaCl	145 mM	2 M (11.688 g / 100 ml)	36 ml
KCl	5 mM	0.5 M (3.728 g/ 100 ml)	5.0 ml
$MgSO_4$	1 mM	1 M (6.019 g / 50 ml)	0.5 ml
KPO_4 buffer pH7.4	1 mM	1 M (11.41 g /50 ml)	0.5 ml
Glucose	10 mM	0.5 M (4.504 g / 50 ml)	10 ml
Hepes	30 mM	1M pH 7.4 (11.915 g / 50 ml)	12 ml
$CaCl_2$ dihydrate	2 mM	0.5 M (3.675 g / 50 ml)	2.0 ml

NH_4Cl buffer: As above but OMIT HEPES and NaCl. Add 25 ml of 1.0 M NH_4Cl (5.349 g / 100 ml) for a final concentration of 50 mM and 23.8 ml of 2M NaCl for a final concentration of 95 mM pH 7.4

Acid Loading Buffer Instructions

Please make either 100 or 50 ml (as shown in the instructions) of each stock buffer in a 100 ml glass culture bottle. The solids are all on the bench in the lab. Label the concentration and name of the stock for each.

These buffers are CRITICAL for using in some of the new cells. Be very very careful when making them.

The stocks do not need to be steril. BUT mix up the buffers first, double check the pH and then filter sterilize. Store buffers and glucose stock at 4°C.

Stocks	
2 M NaCl	(11.688 g / 100 ml)
0.5 M KCl	(3.728 g/ 100 ml)
1 M $MgSO_4$	(6.019 g / 50 ml)
0.5 M Glucose	(4.504 g / 50 ml)
0.5 M $CaCl_2$ dihydrate	(3.675 g / 50 ml)
1 M KPO_4 buffer pH7.4	(11.41 g /50 ml) – this is not PBS. Dissolve the solid KPO4 into 25 ml of water and adjust the pH as needed.
1M Hepes pH 7.4	(11.915 g / 50 ml) - Dissolve the solid into 25 ml of water and adjust the pH as needed
1.0 M NH_4Cl	(5.349 g / 100 ml)

Buffers

Acid Load - Hepes Buffer pH7.4 500 ml

Addition	***Final Conc***	***Stock Addition***	***Check when added***
NaCl	145 mM	36 ml	□
KCl	5 mM	5.0 ml	□
$MgSO_4$	1 mM	0.5 ml	□
KPO_4 buffer pH7.4	1 mM	0.5 ml	□
Glucose	10 mM	10 ml	□
Hepes	30 mM	12 ml	□
$CaCl_2$ dihydrate	2 mM	2.0 ml	□

Acid Load - NH_4Cl buffer pH7.4 500 ml

Addition	***Final Conc***	***Stock Addition***	***Check when added***
NaCl	95 mM	23.8 ml	□
KCl	5 mM	5.0 ml	□
$MgSO_4$	1 mM	0.5 ml	□
KPO_4 buffer pH7.4	1 mM	0.5 ml	□
Glucose	10 mM	10 ml	□
NH_4Cl	50 mM	25 ml	□
$CaCl_2$ dihydrate	2 mM	2.0 ml	□