

Xenopus electrophysiology solutions from Dr. Arseny Khakhalin

External Solution

Recipe

These substances are to be added to the stock from the very beginning.

What	Molarity, mM	g/l (ie. for 1l of solution)
NaCl	115	6.7206
KCl	4	0.298
HEPES	5	1.191
Glycine	10 μ M	1ml of 10 mM stock
Glucose	10	1.802

And these 2 substances are added just before the experiment:

CaCl ₂	3	3ml of 1M stock
MgCl ₂	3	3ml of 1M stock

pH = 7.2; Osmolarity = 250

The general tactics is:

1. Take distilled water, ~80-85% of final solution volume, and mix the drugs in;
2. Bring pH to the target, carefully adding suitable bases (acids);
3. Bring Osmolarity to target by adding water.

With amounts shown in the tables, as a result of these procedures, the final solution volume may be slightly (2-5%) less than the target, but the Osmolarity will be correct.

Use a 1l jar with a magnetic stirrer working at ~300 rpm ongoing. Use big hexagonal weighboats for NaCl, and sheets of thin paper for everything else. To transfer the stock solutions, use 1000ml pipette with a blue button, and blue conical tips. While pressing the button, 1st stop measures the volume; 2nd stop empties the pipette entirely, 3^d ejects the tip. Beware that the scales take long time to switch on for the 1st time, and they can't zero the TARE as long as the weight is not stable (eg. the window is open, or you are jumping nearby creating vibrations).

After the Osmolarity is measured, and the stock is brought to its final volume, it should be filtered with a vacuum-driven huge orange non-reusable filter right into a bottle where it will be stored. Label the bottle, indicating owner, date, and type of solution.

Before the experiment, Mg and Ca are added from stock solutions, using a measuring pipette. The tradition is to add Mg first, and Ca second, just to ensure that everybody always do it in the same sequence, so that even after being distracted, you don't need to recall what exactly drug you have already added, but you can just follow a simple rule.

pHmeter

1. Switch on
2. Move the sensor out of the little jar with yellow buffer
3. Open a hole on the side of the sensor upper part, rotating a violet ring
4. Rinse the sensor with dH₂O so that the yellow buffer doesn't spoil our solution
5. Immerse it into our rotating solution
6. Wait while pHmeter writes "Stable"
7. Add a drop of 10N base (NaOH for external, KOH for internal). Use plastic pipette with a sharp tip
8. Repeat 6-7 until you are ~1 pH below the target, then switch to 1N base and continue
9. If an overshoot – correct with HCl, and repeat 8 if necessary
10. When the solution is fine, make steps 5-1 backwards, returning the pHmeter in its initial state

Osmometer

1. Move black lever at the right side towards you
2. Carefully pull the black thing out, so that a shiny plate is seen
3. Take a small paper disk from a box with a forceps, and place it carefully on a shiny plate, centered
4. Take a black pipette, put a tip on it (it has its own tips)
5. Move a drop of solution (as much as the pipette takes actually) to the disk, placing pipette in a notch, ensuring that the disk is still centered
6. Without hesitation carefully push the thing into the apparatus
7. Move the lever back from you
8. The apparatus starts to countdown. After the countdown it will say the osmolarity. Meanwhile you can remove a tip from the pipette by lifting (rather than pressing) a radial lug up
9. After the measurement, open the thing again, remove the disk, carefully and gently wipe the plate, add a drop of distilled water, wipe it again so that it is dry, push the thing back, and close the right lever. The apparatus will start the countdown again, and if the plate is clean – it will go into standby (=READY) mode. If it measures anything – then the plate was not clean and dry, and needs to be re-wiped.
10. From time to time the Osmometer may need calibration. Solutions for calibration are available, and naturally that one should be used, which has the closest Osm to our target Osm.

Internal Solution

Recipes

For the internal, these substances are to be added to the stock from the beginning:

What	Molarity, mM	g / 100 ml of solution
K-gluconate	100	2.3430
KCl	8	0.0596
NaCl	5	0.0292
MgCl₂ · 6H₂O	1.5	0.0305
HEPES	20	0.4766
EGTA	10	0.3804

Target pH = 7.2; Osm = 250

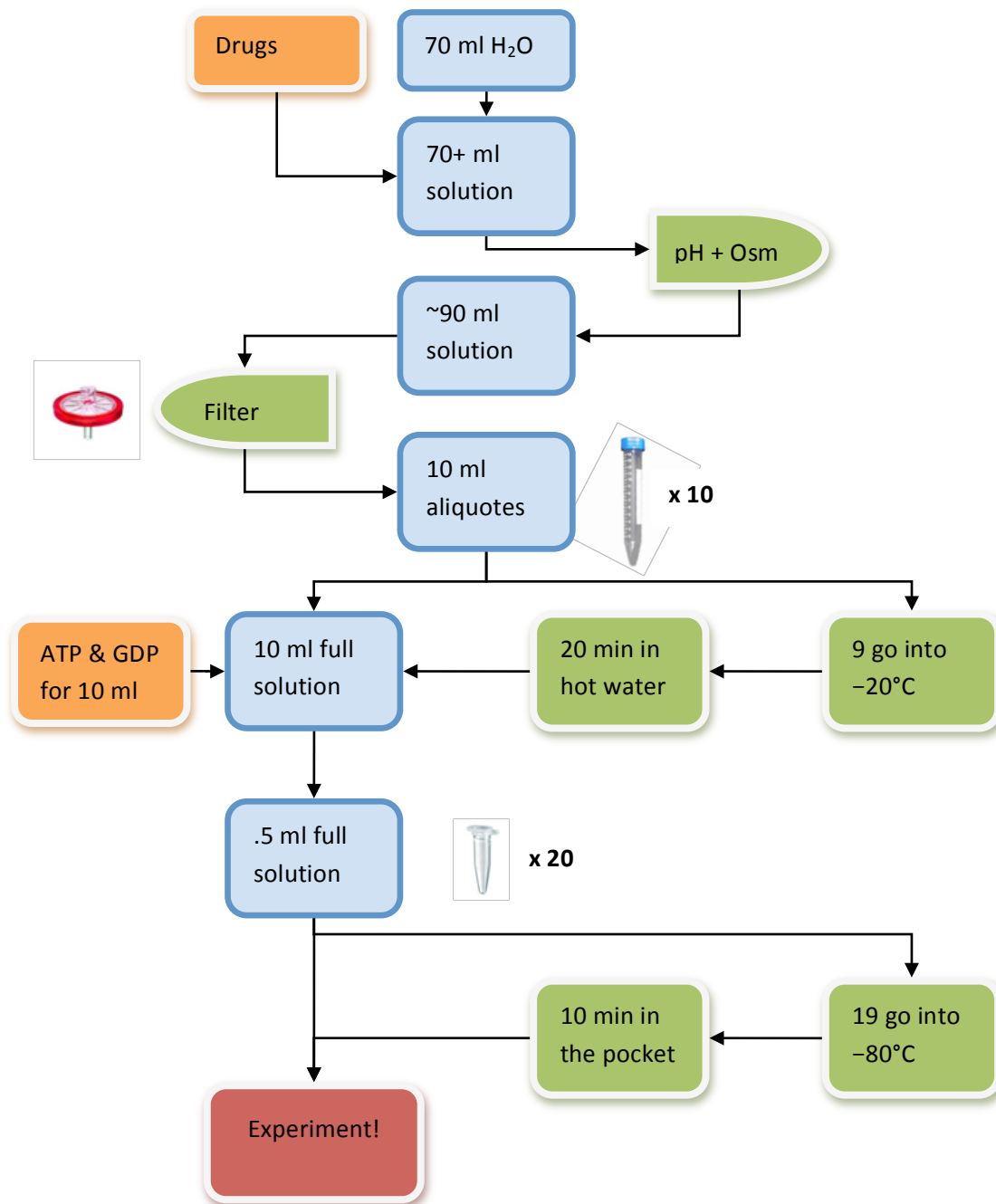
And these 2 substances, being unstable, are prepared and added separately, as it is described below:

ATP	2	11 mg / 10 ml
GTP	0.3	1.57 mg / 10 ml

Procedure

1. We first try to make 100 ml of internal solution without ATP and GTP. Start with ~70 ml of water, and add all the required drugs.
2. Drive the solution pH to the target (7.2), using KOH as a base (HCl in case of overshoot).
3. Measure Osmolarity, and add water to make the stock 250 ± 5 Osm. We should usually end up with 90+ ml of solution.
4. Aliquot in 10 (or 9) tubes 10 ml each, using a syringe, and filtering the solution at the same time (draw without a filter, expel through the filter). All tubes except one are to be frozen at -20°C .
5. Separately make ATP and GTP for 10 ml of solution, and add to the single tube that goes into further processing. Use small hexagonal weighload, and when the desired weight is achieved, take 200-300 μl of solution from a tube with a micropipette, and move it to the weighload; dissolve the grains, and move the solution back. To dissolve the grains quicker, you may try to suck and expel the liquid with the micropipette (same tip) several times. If you have problems with measuring the required weight precisely, you may also weigh slightly more, then move some volume as described, and then move back only part of the volume, proportional to the ratio of desired weight to actual weight, thus ensuring correct concentration in the solution.
6. Aliquot this full solution into 20 2.5 ml conical tubes, .5 ml in each. 19 of them go into deep freeze at -80°C , while one goes into experiment. The fridge is located in the lab nearby.
7. To unfreeze a full stock .5 ml tube, just remove it from a deep fridge, and put it in the pocket for several minutes. To unfreeze the 10 ml of ATP/GTP-less stock, keep it in hot water for ~20 min, applying vortex shake from time to time.

Internal Solution Flowchart



Predicted properties for these solutions

EK	-102 mV
ENa	72 mV
ECl	-53 mV
Junction Potential	14 mV