Orca 4 Order of Operations - CTD Sampling

1) Retrieve CTD - 12x 12 L bottles total
2) Identify and attach appropriate sampling tubes/filtering apparatus
3) Start wet sampling in order - get through red asap, then continue as able:
    a) Methane - unfiltered **60 mL**
    b) Whole cells **~1 mL**
    c) Attach Acropak 0.2 um filters, flush
    d) Sulfide - filtered **1 big 60 mL; 2 small 10 mL samples**
    e) Spec syringe **1 60 mL syringe**
    f) DIC **Single**
    g) pH **Duplicate 12 mL exetainer**
    h) Alkalinity **Single**
    i) Nutrients **Single 50 mL falcon**
    j) fDOM **Single 20 mL amber**
    k) d2H **Single exetainer**
    l) Salinity **Single 15 mL falcon**
    m) dt metals **Single 50 mL falcon**
    n) **Collect remaining water into acid cleaned baritainers and take inside to filter via peristaltic pump with acid cleaned tubing and filter holders**
    o) Start filters:
        i) 2x 0.7 um GF/F, fired
            (1) With GF/F, sample DOC 30 mL into VOA vial, fired
        ii) 1x sterivex

At some point:

4) Collect extra Orca Basin brine, filtered at 0.2 um - either acropak off of a completed Niskin bottle or peripumped out of a baritainer
5) Collect ~15 L of Orca Basin brine, unfiltered, directly from Niskins to trilaminate bags for anoxic storage of live water

Additional sample treatments

6) ASAP, fix cell samples with paraformaldehyde. 850 mL sample + 150 uL 20% PFA
7) When available, acidify 30 mL DOC samples with 60 uL clean 4 N hydrochloric acid
8) Transfer 1 mL of filtered sample from salinity falcon tube into 2x 2 mL tubes and 1x GC vial for IC, $BaSO_4$ precipitation, carboxylic acids
    a) IC and $BaSO_4$ acidified with 10 uL 6 N HCl, except for Cast 1 = 2 uL

Sulfide - Zinc acetate precipitates

Materials:
250 ml HDPE centrifuge tubes + 100 mL 10% ZnAc
15 mL falcon tubes + 4 mL 10% ZnAc x2
60 mL syringe
2.5” cannula needle. Ideally 16 gauge but 22 gauge can be used if you want to be miserable

Preparation:

1) Make 10% Zn-acetate solution (probably better to use 5%...we just had things weighed out to be easier for 10% this time).
    a) Sparge Zn-acetate
    b) Fill up 250 mL tubes to 100 mL
    c) Fill up 15 mL falcon tubes to 4 mL
    d) Before sampling, top off tubes with argon

Sampling:

1) Connect clean/dry tubing to Niskin bottle with an appropriate connection to your acropak (we have both barb and NPT backed filters) let tubing flow for a bit to pre-rinse
2) Attach acropak
3) Fill up and rinse a 60 mL syringe with filtered sample x3
4) Refill syringe and connect long cannula, take care to not have air pockets
5) Add 60 mL to big centrifuge tube through cannula into the lower end of the sparged Zn-acetate
6) Collect >20 more mL of filtered sample
7) Refill syringe and connect long cannula, take care to not have air pockets
8) Add 10 mL to each 15 mL tube through cannula into the lower end of the sparged Zn-acetate
9) Cap and shake samples
10) Wrap caps with parafilm to seal
11) Store cold if possible

Comments:
This procedure is super sensitive to oxygen - try to avoid any exposure of the sample to air. Also, leave the Zn-acetates sealed as long as possible to avoid gas exchange. Shake the samples immediately on capping to get zn-acetate around all the sample water. Zn-acetate precipitates are white and microsized - looks a little hazy. Don’t forget to use the smell-test at the same time - trust your nose!

Seawater Methane Concentrations

Materials:
72 ml Serum vials (fired)
60 mL leur lok syringe
Blue septa - thick, gastight (acid washed +- autoclaved)
Crimp seals (20 mm)
Crimper
NaOH pellets
Long tweezers

Preparation:

1) NaOH transportation considerations: maybe bring two nice, small plastic bottles with some whole pellets within just in case one gets lost - don't use glass! Use HDPE if available - or something else rated for a strong base. Don't ship with acid.
2) Tubing and syringes can be reused, but only if they are cleaned and fully DRY
3) Right before the CTD comes up, prepare the vials per depth by adding two pellets of NaOH into them with long tweezers -
    a) Flush with argon if available
    b) Wash off the tweezers after handling base
4) Have the septa, crimp seals, and crimper ready to go

Sampling:

1) Connect clean/dry tubing to Niskin bottle with an appropriate leur connection to your 60 mL syringes, let tubing flow for a bit to pre-rinse
2) Connect the 60 mL syringe to the tubing and extract 60 mL of water, repeat twice
    a) Otherwise, if easier, pull plunger from back of syringe and let overfill for a bit, then re-attach plunger and adjust volume 60 mL
3) Deposit 60 mL sample directly into vial over the NaOH pellets - be prepared to seal
4) Insert septa and crimp to seal
    a) Can wet the stopper with a bit of sample water if it is giving you a hassle to get in - twist it around!
5) Store securely, upside down. These do not need to be chilled or frozen - but they do need to be safely packed! Glass likes to crack, cracks like to leak gas
    a) I make little individual bubble wrap packs for them

Additionally:
Don't forget to make sampling blanks. I prep a 60 mL syringe with mQ water, put a couple NaOH pellets in an extra serum vial, then wave around the serum next to the rosette to get an idea of the background methane level on the boat that may be getting into the samples. After wafting about, inject 60 mL mQ over NaOH for volume consistency, seal and crimp.

Ferrous and Ferric iron by ferrozine assay sampling

Materials:
15 mL Falcon tubes with 0.25 mL of 10 mM ferrozine sol'n pre-aliquoted
Set of acid washed and mQ rinsed sampling syringes with leur lock stopcocks
Barb to leur M adaptor

Preparation:

1) Make 10 mM ferrozine, 1.4 M hydroxylamine hydrochloride, and ammonium acetate solutions
2) Clean plasticware
    a) mQ rinse 3x
    b) Acid wash for 24 hours or more
    c) Fill with mQ water completely 5x
    d) Fill with mQ water for 24 hours or more
    e) Let sit to dry
3) Aliquot 250 uL of ferrozine into each falcon tube
4) Close tubes and keep in the dark

Sampling:

1) Using stopcock system, rinse syringe and collect air-free sample
    a) Twist stopcock so the sample flows into the syringe. Fill the syringe completely
    b) Twist stopcock so the connection is between the syringe and the unattached "drain" side. Push the rinse water held in the syringe through the drain, then twist the stopcock back to "niskin-syringe" position
    c) Repeat this cleaning process once more
        i) The goal here is to rinse the syringe with sample water without exposing the intake to atmosphere
2) Collect sample
    a) Fill sampling syringe completely, making sure that there are no air bubbles in the syringe or stopcock because the rinsing process removed them
    b) Close stopcock and remove from Niskin line
    c) Open labeled eppendorf vial
    d) Transfer 2.5 mL of sample to ferrozine tube, seal, and swirl
3) Measure in the next ~30 mins

    Additionally:
    Transferring ferrozine at sea has issues with stock contamination because boats aren't clean spaces

Analysis
UV-Vis

Ferrous and Ferric iron by ferrozine assay measuring

Materials:
P200 and tips
UV-Vis
1 cm pathlength cell
2x 3 mL syringe, 1-2x/sample
Green 20 gauge needles for sample transferring, 2x/sample
1.4 M hydroxylamine hydrochloride solution
10 M ammonium acetate solution
Watch or other thing to track time
Sampling notebook
Matrix water - Orca basin brine that has been in the dark and oxic for > 1 week

Measurement:

1) Turn on the UV-Vis about 30 mins before use. Inject ~3 mL of matrix water and blank it. Scan again and blank again, until the blank looks good (normally distributed around 0 with a magnitude less than or equal to 0.03). Record every scan, blank, what the sample was, and the time!
2) A few minutes after adding 2.5 mL of sample to each, remove exactly 1 mL of sample, including needle volume, and inject it into the UV-Vis. Record what sample this is!
3) Add 270 uL of 1.4 M hydroxylamine hydrochloride to samples that Fe(II) has been measured on. This reduces the Fe(III) to Fe(II). Record the time of this addition! Swirl nicely.
4) After 15 mins but before 1 hour (typically 30 mins tbh), add 100 uL of 10 mM ammonium acetate. This buffers the pH to a range that ferrozine chelates Fe(II) uniformly, as the hydroxylamine hydrochloride sol'n is acidic. Record the time of this addition. Swirly nicely.
5) After 10 mins, inject >=1 mL onto the UV-Vis for measurement of Fe(III)
6) Calibrate the instrument for Fe(II). The calibration for Fe(II) and Fe(III) are the same, so we typically calibrate Fe(II). Nevertheless, to ensure we don't have contamination issues with the Fe(III) reagents, run a blank or two with 5.5 molal sodium chloride

Calibration:

1) Fe(II) oxidizes in air, so it's important to prepare everything before making the secondary stock and transferring it to the standards. If the secondary stock begins to turn yellow
2) Prepare the Fe(II) secondary stock by adding 1 mL of 0.01 M Fe(II) to 9 mL of mQ. I recommend adding 100 uL of 1 N HCl before the Fe(II) addition to stabilize it better

Standard	0	1	2	3	4	5	6
Matrix/uL	1000	980	975	965	960	950	930
1 mM Fe2	0	20	25	35	40	50	70

10 mM ferrozine	100	100	100	100	100	100	100

DIC Sampling

Materials:
20 mL serum vial, acid washed, fired
Niskin connection tubing
Acropak (0.2 um final filter)

Preparation:
1) Vial and septa
2) Crimp seal + crimper

Sampling:
1) Connect clean/dry tubing to Niskin bottle with an appropriate connection, to your acropak (we have both barb and NPT backed filters) let tubing flow for a bit to pre-rinse
2) Attach Acropak
3) Fill DIC vial out of acropak from the bottom
4) Set septa on vial, shake to rinse…repeat 2x
5) Refill vial and let overflow for a 2x volume increments if water budget allows
6) Set septa and seal ASAP
7) Store cold, not frozen

Comments:
These have to be measured pretty fast, since we aren't killing with any type of poison.

pH Sampling

Materials:
2x Exetainer (12 ml)
Niskin connection tubing
Acropak (0.2 um final filter)
Wide bore long needle

Preparation:

1) Make sure your tubing connectors match - will be setting a luerlok needle following an acropak, so the exit tubing needs to have a barb-fe luer attached

Sampling:

1) Connect clean/dry tubing to Niskin bottle with an appropriate connection, to your acropak (we have both barb and NPT backed filters) let tubing flow for a bit to pre-rinse
2) Attach low gauge needle to the filtered water flow
3) Fill exetainer with filtered sample, cap, shake to rinse, and pour out
4) Repeat 2x
5) Fill exetainer with filtered sample from the bottom, through wide gauge needle, let overflow for a few seconds
6) Cap, check for bubbles, try to get air free be having a positive bulge at the top of the water line (or filling the cap a little)
7) Store in the fridge

Comments:
This volume is for pH measurement via UV-VIS - we don't have an electrode that will work with exetainers

Alkalinity

Materials:
20 mL serum vial (acid washed)
Blue stopper (acid washed)
Crimp cap
Crimper
Niskin connection tubing
Acropak (0.2 um final filter)

Preparation:
1) Set out crimp seals and septa

Sampling:
1) Connect clean/dry tubing to Niskin bottle with an appropriate connection, to your acropak (we have both barb and NPT backed filters) let tubing flow for a bit to pre-rinse
2) Attach Acropak
3) Fill alkalinity vial out of acropak from the bottom
4) Set septa on vial, shake to rinse…repeat 2x
5) Refill vial and let overflow for a 2x volume equivalents if water budget allows
6) Set septa and seal ASAP
7) Store cold, not frozen

Comments:
There will be a little bit of air at the top

Nutrients + Total dissolved metals

Materials:
50 mL falcon tube x2
Vinyl copolymer gloves
4-6 N HCl
Niskin connection tubing
Acropak (0.2 um final filter)

Preparation:

1) Make sure if doing open ocean work, or anything with low N, that you aren't wearing nitrile gloves! That will be contaminating. We typically use clear vinyl copolymer gloves.

Sampling:

1) Connect clean/dry tubing to Niskin bottle with an appropriate connection, to your acropak (we have both barb and NPT backed filters) let tubing flow for a bit to pre-rinse
2) Partly fill 50 mL Falcons, cap, shake to rinse, and pour out
3) Repeat rinse 2x
4) Collect 1 falcon for nutrients and 1 sample for TDM by filling to 30 mL after rinses
5) For dissolved trace metals, return to ship lab and add 60 uL 4-6 N metal clean acid to pH ~2
6) Freeze samples upright at -20 C

d^2H - water isotopes

Materials:
Exetainer (12 ml)
Water samples
Niskin connection tubing
Acropak (0.2 um final filter)

Preparation:
1) Nothing special needed

Sampling:
1) Connect clean/dry tubing to Niskin bottle with an appropriate connection, to your acropak (we have both barb and NPT backed filters) let tubing flow for a bit to pre-rinse
2) Fill the exetainer with filtered sample part way, cap, shake to rinse, pour out
3) Repeat rinse step 2x
4) Fill exetainer with filtered sample - slightly overfill both tube and cap with water then join quickly to collect air-free sample
5) Store in the fridge

Comments:
These are pretty stable measurements, the big thing is to just not let them evaporate. And also, for the same reasons, I don't really recommend freezing them unless for long term storage as the freeze/thaw combo can be wacky on the volumes/evap if repeated too often.

fDOM

Materials:
20 mL amber glass vial (fired)
20 mL cap with teflon liner (acid washed)
Water samples
Niskin connection tubing
Acropak (0.2 um final filter)

Preparation:

1) If you don't have amber glass, spend some time wrapping clear combusted glass in aluminum foil
    a) The goal here is to have samples not exposed to light

Sampling:

1) Connect clean/dry tubing to Niskin bottle with an appropriate connection, to your acropak (we have both barb and NPT backed filters) let tubing flow for a bit to pre-rinse
2) Fill the amber glass vial with filtered sample part way, cap, shake to rinse, pour out
3) Repeat rinse step 2x
4) Fill up amber glass vial with ~20 mL filtered sample
5) Cap and parafilm
6) Store in fridge

Comments:
Don't freeze these!

IC - major ions; $BaSO_4$ - precipitated sulfate

Materials:
2 mL centrifuge tubes
p1000 + tips
p10 + tips
Collected filtered water samples
6 N HCl
$BaCl_2$ ~ 1 N (a concentration matching or exceeding expected final sample concentration

Preparation:
1) For better results and dilutions, we've moved away from sampling directly off of the Niskins to aliquoting out of 15 mL Falcon tubes filled with 0.2 um filtered seawater samples
2) Need to have prepared 6 N HCl and 1 N BaCl solutions around

Sampling:
1) Pipette a 1 mL aliquot of sample from Falcon tube to separate labeled 2 mL tubes
2) Pipette 10 uL of 6 N HCl into each sample
3) Cap and shake to mix!
4) IC sample is done
   BaSO4 is good to acidify - if - there is a potential for free H_2S acidifing will flush that out as a gas…otherwise, it will likely oxidize to sulfate and get bound into the sulfate pool, which we don't want!
5) For the precipitated $BaSO_4$ sample, wait 15+ minutes after acid addition (covered from dust, maybe in fume hood/flow bench if available)
   a) Then, pipette an equivalent amount of BaCl solution to the filtered seawater sample
   b) Shake to mix - a white precipitate will form - this is $BaSO_4$
6) Freeze or fridge the IC sample - for long term storage should probably be frozen; $BaSO_4$ is fairly stable, but normally fridge them anyway

Comments:
Freezing brine is not ideal due to the high amount of salt...they sometimes also don't redissolve properly. Also, the addition of HCl will throw off any chloride measurements. If you want a chloride measurement out of the IC samples, you'll need to take a second aliquot out of the salinity master tube for it. If there is no chance for sulfide, I probably wouldn't acidify the precipitated sulfates before adding barium chloride (in this case I normally pre-aliquot BaCl as part of the prep to cut down on after work).

Carboxylic Acids

Materials:
2 mL GC vials (fired) + caps
p1000 + tips
Collected filtered water samples

Preparation:

1) For better results and dilutions, we've moved away from sampling directly off of the Niskins to aliquoting out of 15 mL Falcon tubes filled with 0.2 um filtered seawater samples

Sampling:

1) Pipette a 1-2 mLsample aliquot from prepared Falcon tube to a GC vial
2) Freeze!

Comments:
Freezing brine is not ideal due to the high amount of salt...they sometimes also don't redissolve properly.

Water Column DNA

Materials:
Peristaltic pump
Peripump tubing to luerlok connection (acid washed)
Sterivex filter
Whirlpak
Baritainer (or other acid washed graduated container)
Optional: big graduated cylinder

Preparation:
1) Everything needs to be sterile. In lab, this is easy - on boats a little more complicated, but you do the best you can and take blanks when needed to compare.
2) I typically resort to a 3x 5-10% HCl rinse and shake for containers, and a 3 min HCl rinse for tubing, followed by 3x mQ rinses or 3 min of pumping mQ
3) Try to make a sterile workspace and think clean thoughts for best results

Sampling:
1) Rinse out your water container with sample
    a) There should be a acid-clean/sterile container to store water in already, but we are going to increase our success rate by filling it a little with live sample water, swishing it around vigorously, and pouring out - 3x
2) Fill water container up to desired amount and take to your peri pump
3) Start the pump slow, rinsing the (already clean) masterflex tubing with sample analogous to the container swish and rinses
4) Pop open a sterivex pack and sharpie label on the sterivex body to match the sample label and date
5) Attach sterivex filter to the rinsed sample tubing line via a luerlok connection (sterivex fit on female connectors)
6) Begin pumping water through the sterivex at slow-medium, watch for pop-offs due to pressure
    a) Can use a big graduated cylinder or similar to catch flowthrough to get a second amount estimate
7) Continue for x L (typically 1-2) or until sterivex clogs
    a) When complete, record the water amount that passed through the filter
8) Promptly place sterivex into a labeled whirlpak and freeze at -80 (or as cold as possible)

Comments:
Sterivex are ~ 0.2 um filters - you can use the filtered water for stuff if you want. I usually just go off of my big whole water reservoir for literage passed through, since we do multiple filters at a time. Need to know the overall amount of water filtered for later comparison! On longer trips I will alternatively bleach soak containers overnight to get a better clean - but need a lot of rinsing - any residual bleach ruins samples. Start with autoclaved containers if possible!

Elemental sulfur / lipids - particulates

Materials:
Peristaltic pump
Peripump tubing to luerlok connection (acid washed)
47 mm filter holder (acid washed)
GF/F filter (fired) 0.7 um mesh (you can go with a finer mesh glass fiber filter if you want, just make sure it is fired)
Fired foil
Whirlpak
50 mL Falcon tube
Argon line
Baritainer (or other acid washed graduated container)
Tweezers
Optional: big graduated cylinder

Preparation:
1) The key here is organic clean. In lab, this is easy - on boats a little more complicated, but you do the best you can and take blanks when needed to compare. If options exist for solvents, cool, but otherwise just be as clean as possible.
2) I typically resort to a 3x 5-10% HCl rinse and shake for containers, and a 3 min HCl rinse for tubing, followed by 3x mQ rinses or 3 min of pumping mQ
3) We don't usually need this level of rigor for our analysis, but oftentimes methanol is used for extra cleaning at sea

Sampling:
1) Rinse out your water container with sample
    a) There should be a acid-clean/sterile container to store water in already, but we are going to increase our success rate by filling it a little with live sample water, swishing it around vigorously, and pouring out - 3x
2) Fill water container up to desired amount and take to your peri pump
3) Flush lines and filter holder with sample water
4) Detach the filter holder and open on a clean surface
5) Carefully take a clean GF/F filter with clean tweezers and install into the filter holder
6) Close the filter holder, making sure all o-rings are set well and all is sealed
7) Reattach the filter holder to sample line and start peri pump
8) Pump desired amount of water through the GF/F - usually 2 L
9) Remove the GF/F and wrap in clean, fired foil…store in a labeled whirlpak
10) Repeat sampling with a new GF/F, store this one in a Falcon tube under an argon atm
11) Freeze both GF/Fs and record the amount of water filtered

Comments:
Usually, I just do this filtering in sequence with DNA out of the same overall baritainer with the same peripump sample tube. During the GF/F filtering is when I take a DOC sample! GF/F filters

are ~0.7 um mesh size, so you can really crank up the peripump to pretty fast flows. Keep an eye on the filter holders to make sure they don’t start with or develop leaks.

Dissolved Organic Carbon (DOC)

Materials:
Peristaltic pump
Peripump tubing to luerlok connection (acid washed)
47 mm filter holder (acid washed)
GF/F filter (fired) 0.7 um mesh
40 mL VOA vial (fired)
Teflon lined vial cap (cleaned)
p200 + tips
Baritainer (or other acid washed graduated container)

Preparation:
1) The key here is organic clean. In the lab, this is easy - on boats a little more complicated, but you do the best you can and take blanks when needed to compare. If options exist for solvents, cool, but otherwise just be as clean as possible.
2) I typically resort to a 3x 5-10% HCl rinse and shake for containers, and a 3 min HCl rinse for tubing, followed by 3x mQ rinses or 3 min of pumping mQ
3) The GF/F sample setup

Sampling:
1) While filtering onto a GF/F, use some of filtered flow through effluent to partly fill a VOA vial - cap, shake and rinse, pour off
2) Repeat 2x
3) Fill the VOA vial to 30 mL with GF/F filtered sample water
4) Acidify with 60 uL 4 N clean HCl
5) Cap, parafilm, store 4-20 C in a location without organics

Comments:
These should be refrigerated and can be frozen. Transport with care. DOC is pretty easily contaminated so make sure you are using proper tubing/setup and are not adding DOC from random sources throughout the process.

Anoxic whole water

Materials:
Some kind of clean large sample holding bottles - Glass media bottles with thick septa would be good, but pretty bulky. We are using trilaminated aluminum gas sampling bags with sealing closures for ease of transport, ect

Preparation:
1) Autoclave everything you can before packing...otherwise can clean with acid or bleach
2) Degas anything rubber by leaving in a N2 or argon atmosphere
3) Purge the interiors of any holding/transport vessel you're using

Sampling and transport:
1) Connect clean tubing to niskins, flush for a bit
2) Connect tubing directly to trilaminate bag intake, open valve, let fill
    a) If using a sealed media/serum bottle, fill with argon or n2 then fill unfiltered from niskin with a double needle (water in/gas out) technique
3) Seal and close
4) Put samples iin a secondary reducing atmosphere bag (usually just fill something with argon) and transport chilled

Comments:
Probably best to coarsely filter before starting incubations to remove any larger particulates…but no need to do that for transport.

General Cell Fixation - From monoFISH Prep

4% PFA recipe – do this before you need it

> *PFA is gross and denatures to formaldehyde with heat. Do the prep steps in a fume hood. When working - all PFA contaminated items should go into an individual PFA waste. PFA contaminated liquids should go in a combined PFA waste tube.*

1. Heat 33 ml mQ H_2O to 60°C in a 50 ml tube.
2. In the fume hood, add 2g paraformaldehyde and a drop of 2N NaOH. Close 50 ml tube and mix to dissolve. Add an additional drop of 2N NaOH if this takes more than 2 minutes.
3. Add 16.5 ml 3X PBS or 4.95 ml 10X PBS, cool and adjust to pH 7.2. Bring the final volume to 50 ml with H_2O (this can all be adjusted for samples needing higher salt concentrations by making up 4% PFA in 3X PBS instead of 1X).
4. Sterilize with 0.22 µm filter and store the 4% PFA as 2 ml aliquots at -20°C.

To save on hassle, just buy ampoules of 20% PFA and dilute as needed.

General cell fixation in PFA

1. In a 2 ml tube, take ~0.1 g crushed carbonate or 1 ml culture or 1 ml sediment slurry. Centrifuge fast to pellet cells/sediment, decant supernatant and resuspend in 250 µl 3X PBS. Vortex/mix to fully suspend.
2. To slurry mixture add 750 µl 4% PFA. Invert tube to mix and incubate at 4°C for 1-3h. Leave the incubation longer the more challenging the substrate is to totally fix.
3. Centrifuge at max speed for 3 min to pellet. Remove the supernatant into a dedicated PFA waste.
4. Wash the pellet with 3x PBS to remove the remaining PFA (any volume is ok), pellet via centrifuging, pour off supernatant, and resuspend in 3x PBS. Repeat 2 more times. Finally, resuspend in 1:1 PBS:ethanol (200 µl final volume should be good, but use any volume needed to achieve desired cell density). Use higher concentration PBS for more saline samples (to prevent osmotic lysis). For hypersaline samples ethanol will crash out the salt, so just use very salty PBS.