Reagents for MACS OPC Isolation – JB, updated by AES 1/26/16

PDL/Laminin Coating Plasticware

PDL

100x PDL stock (1 mg/ml) – Add 5 ml sterile dH2O to a 5 mg bottle of poly-D-lysine (Sigma P6407) in the hood. Leave at +4 overnight, making sure the bottle stays upright. The next day, gently mix the contents by swirling. Make 0.5 ml aliquots in CRYOVIALS. Store at -80 C. Each aliquot can be dilated in 50 ml sterile dH20 to make 1x PDL (5 ug/ml). 1x PDL can be used to coat plates once, then must be thrown out.

Coating:

Add 1x PDL to plates/dishes. Incubate in hood for 1-3 hours. Aspirate the PDL, wash 3x with sterile dH2O. Dry, with the lids partially open, at the back of the hood for 20-30 minutes or until completely dry. The coated plasticware can be laminin coated immediately, or stored at +4C.

Laminin

Thaw 1mg vial of commercial laminin (Sigma L2020-1mg). Add to 50 ml sterile DPBS to make a 20 ug/ml working stock.

Coating:

Add appropriate amount (6-10 ml for 10 cm dish, etc.) to PDL coated plates/dishes. Incubate at RT in hood or 3 hours or longer. The laminin can be reused once, so be careful to keep it sterile. Add the used laminin solution to a fresh 50 ml conical if this is its first use; discard if it has already been used. To keep track of whether or not laminin has been used already when coating plates/dishes, I label the plasticware with “L1” or “L2” to designate whether it is the first or second time it has been used. Rinse the plasticware 1x with sterile DPBS and dry dishes with lids partially open in the back of the hood. Dishes are sufficiently dry when there is a visible opaque film covering the surface.

I prefer laminin coating dishes on the day of use, but can also wrap dishes in parafilm and store at +4 if necessary.

MACS Buffer

To Make MACS Stock Solution

DPBS-G	500 ml
0.5 M EDTA (Sigma E7889-100ML)	0.5 ml (0.5 mM)
Phenol Red (Sigma P0290)	4 ml

Filter sterilize and keep at +4 C or room temp.

Complete MACS Buffer (Working Solution)

(Before isolation procedure, make sufficient complete buffer for that day)

MACS stock solution	36 ml
5% BSA aliquot	4 ml
400X Insulin (one insulin aliquot)	100 ul (5 ug/ml)

pH to 7.4, filter sterilize and store on ice (or at +4 C if making the previous day)

DPBS-G

Regular DPBS made from the powder (Gibco 21600-010), just include 1g of glucose (Sigma G-5400) per liter. pH to 7.4 before filtering.
Store at +4 C or RT.

5% BSA Aliquots in DPBS

Make 40-50 ml at a time. Add the required amount of BSA (e.g., 2 g BSA in 40 ml) (Sigma A8806) to half the final volume of DPBS. Be careful when mixing – BSA froths. Dissolve gently for ~30 minutes on rocker at +4 C, add the rest of the DPBS and again dissolve gently on rocker. pH to 7.4. Filter through 0.22 um Millex filter, aliquot, and store at -80 C.
5% BSA stock is used frequently in MACS buffer (see above) 4 mls at a time, and in mOPC medium, 1 ml at a time. As such, make both 4 ml aliquots and 1 ml aliquots.

Insulin Aliquots (2 mg/ml; 400X)

To 5 ml sterile water, add 25 ul 1N HCl. Add this to 10 mg insulin (Sigma I-6634). Mix by gently rocking. Filter through 0.22 um Millex filter.
Make 100 ul aliquots and store at -80 C. Each aliquot makes 40 ml 1X insulin (5 ug/ml).

mOPC-A Medium

Make DMEM-F12 medium WITHOUT HEPES from powder (Gibco 12500-062). Ignore instructions on the package. Dissolve in 950 ml MilliQ water. Add 2.1 g/L $NaHCO_3$ (Sodium Bicarbonate – Sigma S5761-500G). Adjust pH to 7.1, add remaining 50 ml water, and filter with a bottle top filter into 2 500 ml bottles. Add the following:

DMEM-F12 500ml	
N2 (Gibco 17502-048)	5 ml (1%)
B27 (Gibco 17504-044)	10 ml (2%)
Pen-Strep (Gibco 15140-122)	5 ml (1%)
5% BSA	1 ml aliquot (0.01%)

Filter sterilize and aliquot – 40 ml each into 50 ml conical tubes. Store at +4 C.

***pH of mOPC-A medium (especially once 2X growth factors are added) will tend to shift towards basic levels at +4 C. Be aware of this and pH with sterile 1N HCl as necessary.**

mOPC-A Medium with 2X Growth Factors

To 100 ul mOPC-A medium, add the following concentration of growth factors:

FGF-2	4 ug, or 400 ul aliquot (40 ng/ml final)
PDGFaa	2 ug, or 200 ul aliquot (20 ng/ml final)

*For 40 ml aliquots of mOPC-A medium:

FGF-2	1.6 ug, or 160 ul
PDGFaa	0.8 ug, or 80 ul

Growth Factor Stocks

Make all stocks in sterile DPBS + 0.2% BSA (24 ml DPBS, 1 ml 5% BSA) at a concentration of 10 ul/ml. Prelabel a sufficient number of sterile 1.5 ml tubes – write the growth factor name, actual amount in ug, and volume in ul on the tube.

The following steps should be done under sterile conditions:

NOTE: Take extreme care not to allow the solutions to froth when dissolving or aliquoting. This will denature the protein and reduce activity.

Allow the growth factor to warm up to room temperature and then spin down for 1 minute at 4000 rpm (if in a bottle) or briefly at 14,000 rpm in the microcentrifuge (if in a small vial). In the hood, add 1 ml DPBS-BSA gently. Seal and keep on ice for 10 minutes. Check to make sure the powder has dissolved. Aliquot as following:

PDGFaa (Sigma P3076-10UG) – comes in 10 ug lots. Aliquot 2 ug (200 ul) into 5 tubes.

FGF-2 (Millipore GF003) – comes in 50 ug lots. Add more DPBS-BSA to a final concentration of 10 ug/ml (transfer the 1 ml to a 15 ml tube, then wash out the bottle with 1 ml of DPBS at a time to a final volume of 5 ml). Aliquot 4 ug (400 ul) into 12 tubes, and 2 ug (200 ul) into 1 tube.

Keep aliquots on ice. Snap freeze in liquid nitrogen and store at -80 C.

O1, O4 Antibodies

We make hybridoma supernatants in the lab. These can be found in the stocks -80 C, bottom shelf. Thaw one tube overnight at +4 C. If not thawed by morning, place in a beaker of cold water until completely thawed. Allow to warm to room temperature (about 30 minutes on the bench).

Working in the hood, add the following to the hybridoma:

O1 or O4	10 ml
5% BSA	1 ml (0.5% final)
0.5 M EDTA	10 ul (0.5 mM final)
2% Sodium Azide	50 ul (0.01% final)

*2% stock of sodium azide is not necessary to sterilize – VERY TOXIC.

Adjust pH to 7.4, filter through a 0.2 um Millex filter and aliquot. You can aliquot 170 ul, which provides enough for two cell preps, but I usually aliquot 85 ul, enough for one cell prep. Label tubes as either "O1 for MACS" or "O4 for MACS." Don't use these for antibody staining. Flash freeze in liquid nitrogen and store at -80 C. Avoid repeated freeze/thaw cycles. The day of the prep, thaw one aliquot in +4 C and mix in MACS buffer (described in prep protocols). If you aliquoted 170 ul, remaining antibody from thawed aliquot can be placed at +4 C for one or two weeks for a future prep.

50 mM B-mercaptoethanol (BME)

The BME (Sigma M7522-100ML) is located in the fume hood in the main lab, and comes at a concentration of 14.3 M. To dilute, add 0.7 ul 14.3M BME to 199.3 ul of MilliQ water, for a final concentration of 50mM.