

# COMPREHENSIVE PEG-PC GEL PROTOCOL

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## MATERIALS

2-Methacryloyloxyethyl phosphorylcholine (Sigma)  
Poly(ethylene) glycol dimethacrylate,  $M_N \sim 750$  (Sigma)  
PBS  
0.22  $\mu\text{m}$  PES syringe filters  
Luer-lock syringes  
microcentrifuge tubes  
vortexer

*For UV curing on coverslips:* Irgacure 2959 (Ciba), a high-intensity UV light source, methacrylate silane-treated coverslips

*For thermal curing:* ammonium persulfate and TEMED (Bio-Rad), vacuum oven/chamber,  $\text{N}_2$  gas, methacrylate silane-treated glass-bottom 96-well plates

*For UV curing on in 96-well plates:* Lithium phenyl(2,4,6-trimethylbenzoyl)phosphinate (LAP, TCI Chemicals), a high-intensity UV light source, methacrylate silane-treated glass-bottom 96-well plate

## PREPARATION OF PRE-POLYMER SOLUTION

1. Make a solution of 20% (w/v) MPC in PBS i.e. 200 mg MPC per mL of PBS (actual w/v after volume change is  $\sim 17\%$ ) and vortex to dissolve. Calculate the volume to make on a basis of 75  $\mu\text{L}$  pre-polymer solution per 18mm coverslip, 50  $\mu\text{L}$  per 15mm coverslip or 40  $\mu\text{L}$ /well in a 96-well plate.
2. Distribute 20% MPC solution into microcentrifuge tubes for each PEG-PC concentration to be made.
3. Add the appropriate amount of PEGDMA. For example, if making 3% PEG-PC and you measured out 300  $\mu\text{L}$  of 20% MPC, add 3% of that volume as PEGDMA i.e.  $0.03 * 300 \mu\text{L} = 9 \mu\text{L}$  of PEGDMA. Mix thoroughly by vortexing. (**N.B.:** If making PEG-PC over 10%, the % of MPC and PEGDMA is calculated differently because the volume changes are substantial).
4. If gels are to be used for cell culture experiments, syringe filter each solution.
5. Degas each solution with ultrapure (grade 5)  $\text{N}_2$  for  $\sim 30$  seconds (this is especially important for thermal curing). Be sure to sterilize the degassing needle with 70% ethanol to keep the solutions clean, and run ethanol through the needle when finished so it does not get clogged.

## POLYMERIZATION ON COVERSGLIPS

1. Make *fresh* 20% w/v Irgacure 2959 in 70% ethanol, vortex to dissolve (it takes a lot of vortexing). Degassing this solution is not usually necessary, but it cannot hurt.

2. Add 40  $\mu\text{L}$  of 20% Irgacure per 1 mL of polymer solution. Mix gently, by inverting or pipetting, so as to not reintroduce dissolved oxygen into the solution.
3. Place 50 or 75  $\mu\text{L}$  aliquots of pre-polymer solution on methacrylate silane-treated cover slips. Carefully cover with an untreated cover slip.
4. Treat with UV at 2-3" distance for 15-20 minutes.
5. Let the untreated coverslip float off in PBS overnight. You may need to gently remove it with forceps the next day.

### **POLYMERIZATION IN 96-WELL PLATES with APS and TEMED**

1. Make *fresh* 20% ammonium persulfate (APS) in deionized water, vortex to dissolve. Degassing this solution is not usually necessary, but it cannot hurt.
2. To polymerize, add 20% APS to pre-polymer solutions at a 1:80 ratio, and add TEMED 1:800. i.e. 1000  $\mu\text{L}$  pre-polymer / 80 = 12.5  $\mu\text{L}$  20% APS and (1000/800) = 1.25  $\mu\text{L}$  TEMED. **N.B.:** After addition of APS and TEMED it is possible for the solution to polymerize on the bench top, so it is recommended that each PEG-PC concentration be done separately i.e. don't add APS and TEMED to every pre-polymer solution before aliquoting into wells.
3. After addition of APS and TEMED, aliquot 40  $\mu\text{L}$ /well into the wells of a methacrylate silane-treated 96-well plate.
4. Repeat for every PEG-PC concentration being made.
5. Place 96-well plate in vacuum oven at room temperature with the cover off and flow ultrapure (grade 5)  $\text{N}_2$  at 25 psi through the chamber for 5 minutes.
6. After 5 minutes of flowing  $\text{N}_2$ , close the inlet and outlet to the vacuum oven tightly and close the  $\text{N}_2$  valve.
7. After 20 additional minutes, gels should have formed.
8. Swell in PBS overnight. Use 100  $\mu\text{L}$ /well, except for 0.5% PEG-PC gels use 200  $\mu\text{L}$ /well (because they swell so much).

### **POLYMERIZATION IN 96-WELL PLATES with LAP**

1. Make *fresh* 200 mM LAP in 70% ethanol, vortex to dissolve. Wrap the tube in aluminum foil because LAP is light-sensitive.
2. To polymerize, add 200 mM LAP to pre-polymer solutions at a 1:100 ratio. i.e. 1000  $\mu\text{L}$  pre-polymer / 100 = 10  $\mu\text{L}$  200 mM LAP.
3. After addition of LAP, aliquot 40  $\mu\text{L}$ /well into the wells of a methacrylate silane-treated 96-well plate.
4. Repeat for every PEG-PC concentration being made.
5. Treat with UV light (at least 5  $\text{W}/\text{cm}^2$  intensity) for 20 minutes.
6. Swell in PBS overnight. Use 100  $\mu\text{L}$ /well, except for 0.5% PEG-PC gels use 200  $\mu\text{L}$ /well (because they swell so much).