**Preparation of APTES-DSC Glass Coverslips**

**Materials**

18 mm diameter no 2 glass coverslips

Liquid nitrogen

(3-Aminopropyl)Triethoxysilane (APTES)

Mineral oil

Toluene

200 proof ethanol

Nanopure Water

N,N-Disuccinimidyl carbonate (DSC)

Diisopropylethylamine (DIEA)

Dry acetone

Phosphate Buffered Saline (PBS)

12 well Costar plate (polystyrene)

**Procedure**

1. Oxygen plasma cleaning – always make sure that the chamber is free of any debris before using.
	1. *Make sure there is liquid nitrogen in the glass well covered in cloth.*
	2. Place slips in a 12 well plate, 2 per well leaning against each other like this: \/

This ensures that both sides of each coverslip get cleaned without having to do it twice.

* 1. Open the red lever in back, point black lever down in front, push door shut.
	2. When the gauge reads less than 200 mTorr turn the power on, the RF to high and open the oxygen gas valve (yellow) *slightly.* The pressure *will* go up when you open the oxygen gas valve, just keep the pressure at or below 500 mTorr. If it’s working plasma will be reddish purple. Treat for 10 minutes.
	3. To finish, reverse steps: close the O2 valve, turn the RF switch to ‘off’, turn off power, close the red lever and point the black lever up. Leave on the vacuum pump and pump switch.
1. Vapor phase silanization with APTES – perform within a few hours of the plasma treatment!

Tube reactor method:

* + 1. Dry slips and evaporation tube in vacuum oven at 110°C for 10 minutes.
		2. Heat mineral oil to 90°C on hotplate with stir bar.
		3. Measure 0.5 mL of APTES into the tube, transfer coverslips to coverslip holder and place in tube, cap and seal with clamp and then allow to react overnight, 16-18 hours minimum.

Tissue culture plate method:

1. With the coverslips still in the TC plate used for the oxygen plasma treatment, put 100 uL of APTES into the spaces between wells (600 ul total), place the cover on the plate and seal the cover with two layers of tape.
2. Wrap the entire plate in double layer aluminum foil
3. Place in an oven at 90°C overnight, 16-18 hours minimum.
4. Wash coverslips.
	1. Wash in a 600 mL glass beaker with roughly 200 mL of toluene followed by ethanol and then NanoPure water. Blow off excess water with N2 gas.
	2. Dry in vacuum oven, at 115°C under vacuum, for about 1 hour. It is a good practice to flush once with N­2 gas i.e. evacuate the chamber, fill with N2 gas, then evacuate again. This should minimize the amount of water vapor present.
5. DSC treatment.
	1. In a dry 600 mL beaker, combine 0.2 g DSC, 0.67 mL DIEA and 19.3 mL dry acetone. This is sufficient for at least 24 coverslips.
	2. Transfer dried coverslips to the beaker.
	3. Cover the top tightly with double layered aluminum foil, then wrap the entire beaker with double layered aluminum. This is to prevent acetone vapors from getting into the air and possibly damaging incubators.
	4. Shake the solution and coverslips at 100 RPM for ~2 hours at room temperature.
	5. Rinse the coverslips in acetone at least 3 times. Transfer to coverslip rack or an aluminum foil lined surface and dry under vacuum for 1 hour at 115C. Flush the chamber with N2 gas first.
6. Coupling – must be performed in a humid environment to ensure the solution does not evaporate. The following steps can be applied to protein treatment, antibody treatment, or any other surface treatment. **The coverslips surface chemistry will degrade in a matter of hours in an aqueous environment so they must be kept under vacuum or nitrogen until immediately before use.**
	1. Prepare the protein, antibody, etc in PBS.
	2. Place wet paper towels and beakers of water into plastic bin. Place glass plate over wet paper towels and cover with another wet paper towel. Place parafilm on top.
	3. Place 80 uL of solution onto parafilm and place coverslips on top ensuring that the entire bottom surface is covered. These coverslips are very hydrophobic so they will have to be mechanically manipulated to get full coverage. Be sure to keep track of which side is treated.
	4. Cover the plastic bin with a couple layers of aluminum foil to hold in moisture.
	5. Incubate for 30 minutes.
	6. Wash 4 times with PBS.
	7. Use immediately i.e. begin antibody staining, UV sterilization prior to cell seeding, etc.