# 1. Procedure

## 1.1. Materials

1.1.1. PBS (Ca-/Mg-)
1.1.2. Poly-D-Lysine (e.g. Catalog A3890401, Gibco, ThermoFisher)
1.1.3. Cell culture media
1.1.4. Distilled sterile water
1.1.5. Sterile pipettes
1.1.6. Sterile tubes
1.1.7. CytoSort Arrays

## 1.2. CytoSort Array Preparation

1.2.1. To remove the anti-bubble coating and prepare the CytoSort Array for cell seeding:

1.2.1.1. Warm PBS (Ca-/Mg-) to 37°C.

1.2.1.2. Add the appropriate volume of buffer or media to each reservoir of the array (1mL per quad reservoir or 3mL per single reservoir).

1.2.1.3. Incubate at room temperature for 3 minutes.

1.2.1.4. Aspirate the PBS.

1.2.1.5. Repeat this wash protocol twice for a total of 3 washes.

**NOTE: Once prepared, the CytoSort Array must be kept wet. Do not allow the CytoSort Array to become dry as bubbles may form in the microwells, which can negatively impact cell seeding, image quality, and CellRaft release efficiency. Prepped arrays can be stored at 37°C or 4°C prior to coating.**

## 1.3. PDL coating

PDL can be purchased ready to use at a final concentration of 0.1 mg/mL (if PDL is at a higher concentration, dilute to 0.1mg/mL). Add an appropriate volume of PDL to each reservoir to be coated:

**Note: If desired, the seeding insert can be used to reduce volume and ensure coating remains in the footprint of the microwell array.**

1.3.1.1. 500uL PDL/quad reservoir (300uL if using seeding insert)

1.3.1.2. 3mL PDL/single reservoir (2mL if using seeding insert)

1.3.2. Incubate PDL coated arrays at 37°C for no less than 4 hours and up to overnight.

1.3.3. Remove PDL and rinse reservoirs with appropriate volume of distilled sterile water 2 x 3 min washes each.

1.3.3.1. 1mL PBS/quad reservoir (500uL if using seeding insert)

1.3.3.2. 3mL PBS/single reservoir

1.3.4. Remove final water rinse and add appropriate volume of desired cell culture media.

1.3.5. Incubate coated array at 37°C until ready for cell seeding (at least 5 minutes). Remove media wash and seed cells per standard protocol.

**Note: If the seeding insert was used for coating, cells can be seeded either with or without the seeding insert. The seeding insert must be removed prior to performing any scans on the CellRaft AIR® System.**