



Standard Operating Procedure: Manufacture of RosetteArray[®] Plates

Overview: This document provides step-by-step guidance for manufacturing RosetteArray cell culture plates. All steps apply to 6-well, 24-well, and 96-well formats unless otherwise noted.

Equipment Required:

- Tupperware reaction vessels (description below)
- Custom, micropatterned silicon wafers (description below), FlowJEM
- 500 mL Schlenk flask and stopper
- Vacuum pump (or alternative vacuum sources)
- Nitrogen gas source
- 20-way inert gas manifold with flow tubing (description below)
- Glass gas scrubber
- Chemical safety hood
- Biological safety cabinet
- Forceps
- Pipettor
- Scale
- Glass-plate alignment devices (description below)

Materials Required:

- Gold-coated Willow glass sheets (200 μm thickness)
 - 110 mm x 74 mm (96-well)
 - 118 mm x 80 mm, chamfered corners (6-well & 24-well)
 - Schematic below
- 10 mL pipettes
- 5 mL pipettes
- 15 mL conical centrifuge tubes
- 50 mL conical centrifuge tubes
- HS-(CH₂)_m-OC(O)-IzoButyrate-BR, Alkanethiol initiator of ATRP, Prochimia (FT 015-m11)
- Poly(ethylene glycol) methyl ether methacrylate, Sigma (447943)
- Sodium ascorbate, Sigma (A4034)
- 2,2'-Bipyridine, Sigma (1030980025)
- Copper(II) bromide, Sigma (437867)
- Methanol, Sigma (179337)
- Ethanol
- Sylgard 184 polydimethylsiloxane (PDMS) kit, Fisher (NC9285739)
- Adhesive transfer tape, ARcare (90106NB)



- Bottomless well plates:
 - 96-well, Nacalai (CSCSM096-AR)
 - 6-well, Nacalai (CSCSM006-AR)
 - 24-well, Nacalai (CSCSM024-AR)
- Well plate lids:
 - 96-well plate lid with condensation rings, Greiner BioONE (656171)
 - High profile well plate lid, Greiner BioONE (656161)

Protocol Overview:

1. Source glass sheets.
2. Organize gold-coating of glass sheets.
3. Source adhesive transfer tape.
4. Source bottomless well plates.
5. Design and procure silicon wafers for PDMS stamps.
6. Form PDMS stamp(s).
7. Microcontact print glass sheets.
8. Perform ATRP of PEGMEMA on micropatterned glass sheets.
9. Assemble RosetteArray well plates.
10. Package RosetteArray well plates.
11. Perform quality assessment of RosetteArray batch.

Protocol:

1. Source glass sheets

RosetteArray plates are built on 200 μm thick Willow glass sheets. Length and width dimensions vary between 96-well and 6- & 24-well formats.

Glass sheets for 96-well production should be ordered with the following dimensions:

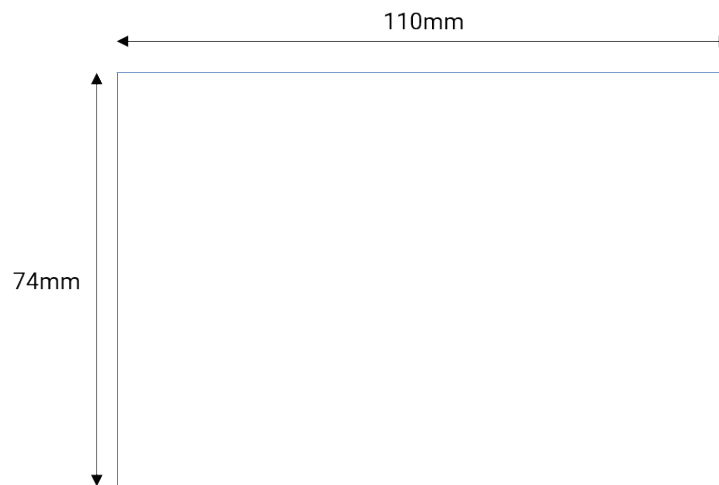


Figure 1.1 96-well plate willow glass footprint should not exceed 112 mm in length and 76 mm in width.

Glass sheets for the 6-well and 24-well production should be ordered with the following dimensions. Note the inclusions of chamfered corners one side of the sheet:

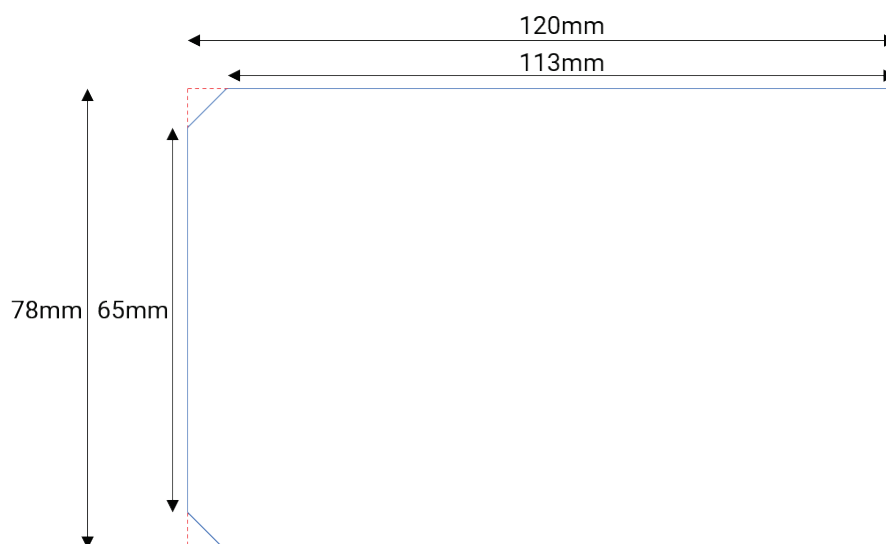


Figure 1.2 6-well & 24-well plate formats require chamfered corners on one side and must not exceed 122 mm length and 80 mm width.

Willow glass sheets may be sourced from the following vendors (listed in order of preference):

- [Coresix Precision Glass](#) (validated, #1 preference)
- [Specialty Glass Products](#)
- [Custom Glass & Optics](#)

Coresix Precision Glass lead time is roughly two weeks, including shipping. Upon receipt, store willow glass sheets in shipping packaging.

2. Gold coat glass sheets

Glass sheets must be coated with a thin layer of Gold (Au) to allow microcontact printing with alkanethiol initiators of ATRP. An adhesion layer of 3.5 nm Titanium (Ti) is necessary for proper binding of and 18 nm layer of Au.

If sourcing Au-coating services commercially it is sufficient to request a 3.5 nm Ti adhesion layer and an 18 nm Au layer. A list of currently validated sources of Au-coating services are listed below:

- [Platypus Technologies](#)

Current agreement with Platypus Technologies is to coat 48 willow glass sheets (of either format) per batch, with 1 week lead time. As there is no convenient way to transport willow glass sheets, transport the samples in the original packaging from the glass supplier and instruct Platypus to reuse the packaging when coating is completed. We do not suggest using commercial shipping for these transport steps.

If commercial Au-coating is unavailable, the Telemark electron beam evaporator in the UW Madison Nanoscale Fabrication Center (NFC) may be used. However, throughput is limited to 20 sheets per 4-hour session. Further, the mounting scheme for glass sheets in the device prevents uniform Au-coating on all but one sheet per run and thus is not recommended for scaled RosetteArray production. When performing Au-coating via this method the protocol is as follows:

Glass sheet preparation:

1. In a chemical fume hood prepare 4 glass Tupperware containers each with 20 mL Toluene, Methanol, Acetone, and Ethanol.
2. Using steel forceps submerge a single glass sheet into the Toluene bath. Gently agitate the bath back and forth, rinsing the glass.
3. Using the forceps to lift the short edge of the glass sheet, remove it from the Toluene bath and place it gently in the Methanol bath. Gently agitate the bath back and forth, rinsing the glass.
4. Repeat steps 2 and 3 for the Acetone bath and Ethanol bath.

5. Upon removing the sheet from the Ethanol bath, use a focused stream of inert gas (Nitrogen) to remove or evaporate ALL liquid. Use may use a gloved hand to hold the sheet along the edges for this step.

NOTE: It is crucial to remove or evaporate all liquid from this step, as sheets are stacked atop one another and any trace liquid will cause them to adhere.

6. Store rinsed glass sheets stacked in between two polystyrene plate lids.

Au-coating glass sheets:

Always follow the NFC guidelines for operation of the Metal Evaporator, i.e., pumping down and cooling between depositions. Outlined here will be the process steps necessary to best ensure uniformity between batches.

1. On the platform stage, (NOT the dome stage) place 5 willow glass sheets on the tool surface using the NFC-supplied low particulate tape in the layout depicted in **Figure 2.1**. Form tape in a loose roll, it should only contact the glass on the BACK surface.
2. Deposit a Ti adhesion layer using a deposition rate of 0.5 \AA/s .
3. Deposit a Au-layer using a deposition rate of 0.5 \AA/s .
4. Store Au-coated glass sheets atop one another

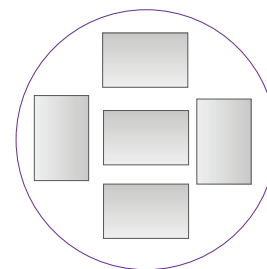


Figure 2.1 Orientation of glass sheets on metal evaporator stage.

NOTE: Deposition rates may vary across sessions (and within sessions). It is normal to see $\pm 0.2 \text{ \AA/s}$, but you should never exceed 1.0 \AA/s .

between two polystyrene plate lids.

Glass sheets coated via this method should be used for microcontact printing within two weeks.

3. Source adhesive transfer tape

Micropatterned glass sheets are fastened to bottomless well plates with adhesive transfer tape. As this tape contacts cell culture media, its critical it not contain reactive or toxic components. This includes innate tape components and artifacts of cutting and processing. The only approved tape for use in RosetteArray manufacture is ARcare 90106NB tape. Additionally, the tape may not undergo any heating (autoclave or laser cutting) prior to assembly. ARcare 90106NB tape may be procured from [Adhesive Research](#) in 30 ft long rolls with 9 in height.

4. Source bottomless well plates

Clear bottomless 96-well and 24-well plates may be sourced from Greiner BioOne, however these require self-application of adhesive transfer tape and manual cutting, either with a scalpel or cutting die. Thus, it is preferable to source bottomless well plates with pre-applied ARcare 90106NB from CS CRIE (a Japanese plate manufacturing firm) through its American subsidiary [Nacalai USA](#). We have special quotes generated for each well plate format we offer. The product numbers are listed below (you may also refer to the materials section):

- CSCM006-AR – Bottomless Microplate, 6-well, with ARcare 90106NB tape
- CSCM024-AR – Bottomless Microplate, 24-well, with ARcare 90106NB tape
- CSCM096-AR – Bottomless Microplate, 96-well, with ARcare 90106NB tape

Nacalai does not offer clear well plates. Lead time for completion of 150 plates is 3 weeks.

Plate lids are NOT included when purchasing from either Greiner BioONE or Nacalai USA. We source the following plate lids from GreinerBioONE:

- 656171 – 96-well plate lid with condensation rings
- 656161 – High profile well plate lid (6-well & 24-well)