

Slide Subbing Recipe & Protocol

Subbing Solution	500 mL	1 L
– Gelatin type A	5 g	10 g
– Chromium potassium sulfate dodecahydrate ($\text{CrK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$)	0.25 g	0.5 g
– MilliQ water (H_2O)	500 mL	1 L

Materials needed

1 L / 2 L beaker	Hot plate
500 mL / 1 L bottle	Magnetic stir bar & retriever
Absorbent bench liner	Microscope slides*
Filter paper	Plastic slide racks (for drying slides)
Funnel	Square glass container(s)
Glass slide racks (for dipping slides)	Thermometer

*Use fisherbrand Premium Superfrost slides. Note, these are not charged. Cat. # 125442

Protocol

1. Add water, magnetic stir bar, and thermometer to a 1 L (or 2 L) beaker.
2. Using a hot plate, heat water to $\sim 40^\circ\text{C}$ with the heat element set low (1 or 2).
 - a. Make sure temperature does not exceed 45°C .
3. Slowly add gelatin to water and stir for 5 minutes or until completely dissolved.
4. Add $\text{CrK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ and stir until completely dissolved.
 - a. $\text{CrK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ will positively charge the slides, allowing them to attract negatively charged tissue sections.
5. Remove the stir bar and thermometer. Filter solution into a 500 mL (or 1 L) bottle.
 - a. Wait for subbing solution to cool to room temperature before use. Solution may be placed in the fridge to speed up cooling process. The solution must be used immediately. Do not store.
6. Place microscope slides into glass slide racks. A box of slides will fill around 8 glass slide racks.
7. Pour subbing solution into a square glass container to $\sim 3/4$ full. If working with another person, fill two containers in order to sub slides at the same time.
8. Dip racks containing clean slides 3 – 5 times (for ~ 5 seconds each) into the subbing solution.
9. Individually move subbed slides into plastic slide racks for drying. A box of slides will fill around 4 plastic slide racks.
 - a. To remove bubbles, slides can be dipped individually into subbing solution. Blot excess solution from a slide by tapping its edge against absorbent liner.
10. Cover racks with paper towel to protect from dust and leave to dry for 48 hours.
11. Transfer dried slides to original slide containers and store at room temperature until use. For slides to be used for cryostat sections, store in the freezer at -20°C .