

Cleaning of coverslips for Total Internal Fluorescence Microscopy (TIRFM)

A. Materials:

1. Coverslip: Erie Scientific 24 x 60 mm, #1 thickness (obtained from Sigma).
The Erie Scientific coverslip proved so far to be cleanest out of the box. Other coverslips tested: VWR 24x60 #1, Corning 24x60 #1, Clay Adams 48x65 #1, Clay Adams 24x60 #0, Menzel-Glazer 24x60 #0. Test was carried out by imaging coverslip surface in air with TIRF (Olympus 60x 1.45 NA lens. Nikon low-fluorescence immersion oil).
2. MilliQ water, >18M Ω -cm, 0.22 μ m filtered
3. Absolute ethanol
4. Acetone, spectroscopic grade
5. 6N KOH, prepared fresh, 0.22 μ m filtered. Note that insoluble potassium-carbonate may form with time in KOH bottle (reaction of KOH with carbon dioxide in air).
6. Nitric acid (HNO₃), concentrated

B. Steps:

1. Place coverslips in glass slide-stainer container. Carry out procedures such that the coverslips remain in the container throughout.
2. Sonicate coverslips in absolute ethanol for 20 min.
3. Rinse 3x with MilliQ water.
4. Sonicate in acetone for 20 min.
5. Rinse 3x with MilliQ water.
6. Incubate for 20 min in HNO₃.
7. Rinse 3x with MilliQ water
8. Incubate for 20 min in filtered 6 N KOH.
9. Rinse 3x with MilliQ water.
10. Store in MilliQ water until further use.

Prior to use:

1. Blow dry with clean N₂ gas.
2. Illuminate coverslips with HBO50 lamp for 30 min to photobleach any remaining background fluorescence.

Coating of coverslips with Ni-NTA for specific immobilization of His6-tagged proteins

A. Materials

1. Cleaned coverslips
2. Toluene
3. MilliQ water, >18M Ω -cm, 0.22 μ m filtered
3. 3-Glycidiloxypropyl-trimethoxysilane (Glymo, Fluka, Cat. No. F50040)
4. N-(5-amino 1-carboxypentyl)-iminodiacetic acid (AB-NTA free acid, Dojindo, Cat. No. A459)

B. Steps

1. Incubate coverslips in toluene vapor containing 2% Glymo for 12 hours at room temperature.
2. Rinse 3x with MilliQ water
3. Incubate in 0.01 M Na₂CO₃ (pH 10), containing 2% (wt/vol) AB-NTA for 16 hours at 60 °C.
4. Rinse 3x with MilliQ water
5. Incubate in buffer containing 10 mM NiCl₂ and 5 mM glycine (pH 8.0) for 2 hours at room temperature.
6. Rinse 3x with MilliQ water.
7. Store in MilliQ water until use.