PEGylation Protocol

as of April 2005
Taekjip Ha's Lab
U of I at Urbana-Champaign

Procedure 1-Cleaning
Step A: microscope slides
Step B: coverslips
Step C: reaction containers

Before Start: Keep used slides in tap water overnight
A-1: Boil the slides in water in a microwave for 5-10 minutes
D-1: Take aminosilane out of a freezer. A couple of hrs at R/T in dark
C-1: Sonicate reaction containers with KOH inside for > 20mins
B-1: Put coverslips in polypropyl containers. Wash them with MilliQ water
B-2: Pour 1M KOH and sonicate for >20 mins
A-2: Take out the boiled slides. Remove coverslips and epoxy completely by a razor blade
A-3: Scrub the slides with MeOH and tap water. Put them in a glass container
A-4: Rinse the container with MilliQ water a few times. Pour 10% alconox and sonicate for 20mins
C-2: Take out the reaction containers from the sonicator. Dispose of KOH and rinse with MilliQ
C-3: Rinse with MeOH and fill with MeOH. Sonicate for > 20mins
A-5: Take the container out and flush with tap water.
A-6: Rinse and fill the container with MilliQ water. Sonicate for 5 mins
A-7: Take the container out. Rinse with MilliQ water
A-8: Rinse it with acetone and fill it with acetone. Sonicate for 15 mins
C-4: Take out coverslip-reaction containers. Dispose of MeOH, rinse with MeOH and dry
A-9: Take the container out. Dispose of acetone. Rinse with MilliQ water
A-10: Transfer slides to polypropyl containers. Rinse with MilliQ water
A-11: Fill them with 1M KOH. Sonicate for 20 mins
B-3: Dispose of KOH and rinse with MilliQ water
B-4: Rinse coverslips with MilliQ water, dry with nitrogen and place in any empty containers
B-5: Burn with propane for ~1sec. Place them in the coverslip-reaction containers (from Step C)
C-5: Do the same thing for the other reaction containers as C-4
A-12: Do the same thing for slides as B-3 and B-4
A-13: Burn them thoroughly for a min. Place them in the slide-reaction container. After A-13, the container is extremely hot
A-14: Cool it down by blowing nitrogen in or let it sit at R/T for half an hour

Procesure 2-Chemical Reaction

Step D: Aminosilanization

Step E: PEGylation

D-2: Pour 100mL of MeOH into the reaction flask. Add 5mL of acetic acid with a glass pipette
D-3: Add 1mL of aminopropylsilane by a glass pipette and mix well
D-4: Pour the mixture in the containers (from part I) and incubate for 10 mins
D-5: For the next use, rinse the used flask with MeOH and MilliQ. Fill with 1M KOH.
D-6: For the next use, dehydrate aminopropyl silane in vacuo for > 15mins
E-1: Put water in the bottom of pipet tip boxes
E-2: Take PEG bottles from a freezer. Put them in dark at R/T
D-7: Sonicate the reaction containers for a min. Then incubate them for another 10 mins
E-3: Make a fresh buffer for PEGylation (10mL MilliQ water + 84mg sodium bicarbonate)
D-8: Seal the aminopropyl silane bottle under nitrogen. Keep in a freezer
D-9: Rinse coverslips with MeOH and MilliQ. Blow by nitrogen and put them in any clean boxes.
D-10: Do D-9 for slides. Put them in the prepared 'water-filled' boxes (from E-1)
D-11: Do D-5 for the reaction containers for the next use
E-4: Take out 1-2mg of biotin-PEG (for five slides). Put it in a 1.5mL eppendorf tube
E-5: Take out 80mg of mPEG (for five slides) and put it in the tube
E-6: For the next use, put PEG bottles in vacuo as D-6
E-7: Add 320uL of the buffer and mix gently by pipette. Spin it for a minute at 10,000rpm
E-8: Mix well (no bubbles). Drop 70uL of it on each slide
E-9: Place a coverslip very gently on the top of the slide (no bubbles)
E-10: Put the boxes in a dark and well-leveled place. Incubate for 2-3 hrs
E-11: After ~10mins, check and restore any slid and misplaced coverslips
E-12: For the next use, treat PEG bottles as done in D-8. Keep in a desiccant bottle at -20C
E-13: Disassemble them, rinse with plenty of MilliQ and dry completely by nitrogen. Assemble the slides according to your application
E-14: Put the assembled slides in a dark and dry place. Suggestion: We use a black-tape wrapped corning tube to avoid light. And 'FoodSaver' to avoid any moisture

Procedure 1 (Sorted)

Before Start: Keep used slides in tap water overnight
A-1: Boil the slides in water in a microwave for 5-10 minutes
A-2: Take out the boiled slides. Remove coverslips and epoxy completely by a razor blade
A-3: Scrub the slides with MeOH and tap water. Put them in a glass container
A-4: Rinse the container with MilliQ water a few times. Pour 10% alconox and sonicate for 20mins
A-5: Take the container out and flush with tap water.
A-6: Rinse and fill the container with MilliQ water. Sonicate for 5 mins
A-7: Take the container out. Rinse with MilliQ water
A-8: Rinse it with acetone and fill it with acetone. Sonicate for 15 mins
A-9: Take the container out. Dispose of acetone. Rinse with MilliQ water
A-10: Transfer slides to polypropyl containers. Rinse with MilliQ water
A-11: Fill them with 1M KOH. Sonicate for 20 mins
A-12: Do the same thing for slides as B-3 and B-4
A-13: Burn them thoroughly for a min. Place them in the slide-reaction container. After A-13, the container is extremely hot
A-14: Cool it down by blowing nitrogen in or let it sit at R/T for half an hour

B-1: Put coverslips in polypropyl containers. Wash them with MilliQ water
B-2: Pour 1M KOH and sonicate for >20 mins
B-3: Dispose of KOH and rinse with MilliQ water
B-4: Rinse coverslips with MilliQ water, dry with nitrogen and place in any empty containers
B-5: Burn with propane for ~1sec. Place them in the coverslip-reaction containers (from Step C)

C-1: Sonicate reaction containers with KOH inside for > 20mins
C-2: Take out the reaction containers from the sonicator. Dispose of KOH and rinse with MilliQ
C-3: Rinse with MeOH and fill with MeOH. Sonicate for > 20mins
C-4: Take out coverslip-reaction containers. Dispose of MeOH, rinse with MeOH and dry
C-5: Do the same thing for the other reaction containers as C-4

D-1: Take aminosilane out of a freezer. A couple of hrs at R/T in dark