

PEGylation Protocol (include DST treatment)

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* This protocol based on the TJ Ha's Lab protocol. I added DSP treatment procedure.

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

B-1: Put coverslips in the reaction (glass) 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 the reaction(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-11: Fill them with 1M KOH. Sonicate for 20 mins

B-3: Dispose of KOH and fill with MilliQ water

B-5: Burn with propane for ~1sec. (only for confocal microscopy experiment)

B-6: Put the coverslips back to the container, fill out with MeOH and sonicate for 10 min

A-12: Dispose of KOH and fill with MilliQ water

A-13: Burn them thoroughly for half a min. Place them back in the reaction container filled with water for rapid cooling.

A-14: fill out with MeOH and sonicate for 10 min

let coverslips and slides sit in MeOH until aminosilanation. Dispose of MeOH right before aminosilanization.

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 and sonicate the used flask with MeOH and 1M KOH. Fill it with MilliQ.

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 (100mM sodium bicarbonate : 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 40mg (for five slides) of mPEG (Laysan bio) and put it in the tube. (for 7 slides, use 56mg mpeg)

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 (for 7 slides, add 448ul)

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

C-0: (Optional) For futher suppression of nucleic acid adsorption, do C1-C7.

C-1: Take out DST bottle and put it in the dark at room temperature 30min before C-2

C-2: Make fresh 100mM sodium bicarbonate buffer (same as E-3)

C-3: Disassemble the pegylated slides and coverslips, rinse with plenty of MilliQ and dry completely by nitrogen.

C-4: Dissolve 3.4mg DST in 20ul DMSO and mix them well by pipette. (for five slides)

C-5: Add 330ul fresh 100mM sodium bicarbonate buffer. Mix them carefully w/ by pipette.

C-6: Do E8-E11, using the DST solution rather than the PEG solution and incubate for 1hr.

C-7: For the next use, treat DST bottles as done in D-6 and D-8. Keep in a desiccant bottle at 4C

E13: 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 coming tube to avoid light. And 'FoodSaver' to avoid any moisture