CHEMICAL REDUCTION OF PORPHYRINS USING PALLADIUM CARBON

Put 5.0mg Pd/C (palladium carbon) and 2.0mg porphyrin in a 13 x 100mm borosilicate test tube. Put the tube on a suitable tube rack.

Wet the contents with 0.2mL water to avoid potential explosion when vapor from the organic solvent comes in contact with Pd/C powder.

Drop a small Teflon coated magnetic flea into the tube.

Perform the rest of this protocol in a fume hood under minimal illumination or darkroom red light.

Minimize exposure to air and oxygen until actual use of the porphyrinogen.

Add 1.8mL methanol and place the racked mixture in a portable polyethylene glove bag (Atmosbag, Sigma-Aldrich, St. Louis, MO).

Replace the atmosphere inside the Atmosbag with hydrogen gas. Keep it fully inflated with hydrogen gas until after the filtration step.

Put the bag on top of a magnetic stirrer and keep the mixture constantly agitated until all UV fluorescence (porphyrin) has disappeared, as tested with a UV lamp. Minimize time of exposure to UV.

Replace the hydrogen with argon. Let the gas flow as needed to keep the bag inflated.

While still in the bag, remove the Pd/C powder via filtration. Transfer the mixture minus the magnet into a syringe with a glass fiber filter. Collect the filtrate into another tube. Cover the tube containing the filtrate.

Remove the filtrate tube from the bag, keeping it under blowing argon. Evaporate the filtrate to dryness while under blowing argon in a water bath at 60°C.

Return the tube containing the dry porphyrinogen into the argon-inflated gas bag and dissolve the contents in about 1mL of the appropriate deoxygenated assay buffer, but with 10mM dithiothreitol added.

Remove a small aliquot, put in 1.5M HCl, oxidize the porphyrinogen with UV and quantify. Adjust the rest of the deoxygenated porphyrinogen solution to the desired concentration.

Always prepare fresh on the day needed.