How to prepare an EPR sample for use in the CalEPR facility

Cleaning of quartz EPR tubes of all sizes:
Prepare all solutions with at least double distilled water (ddH2O), preferably with 18 Ω nanopure water. During each step, please make sure that tubes are filled from the bottom up using a syringe and needle to ensure full solution contact with the entire surface of each tube.

1. Clean any residual sample out of tube using solvent in which previous sample was dissolved.
2. Fill and soak tubes for 12-24 hours in 1M KOH/NaOH to remove protein residue.
3. Repeat filling and soaking with 1M nitric acid overnight.
4. Rinse tubes inside and out with ddH2O, then fill and soak with 4mM EDTA overnight to remove possible metal contamination.
5. Rinse the tubes with plenty of ddH2O, then rinse inside and out with acetone.
6. Dry the tubes in a drying oven for a minimum of 1 hour. Visually check tubes to make sure there is no visible residue. If there is still material evident, repeat entire process from step 1.

Sample preparation and freezing
For sample height considerations, *vide infra*. For a series of sample preparations, all tubes should be filled to the same volume. Sample heights are in addition to the hemi-spherical tube bottom. If there are issues with the amount of sample available, we may be able to work with volumes smaller than this if necessary.

Preferred

X-band 35mm sample height

Q-band 15mm sample height

Minimum

X-band 6mm sample height

Q-band 8mm sample height

For aqueous samples, 30% v/v glycerol or ~0.4 M sucrose can be used as a glassing agent. For samples soluble in organic solvents only, please consult the table of suitable glasses adapted from Drago, et al at the end of this section. Transfer the sample into the EPR tube using either a syringe or a long glass pipette to prevent loss of the sample on the walls of the tube.

Freezing is done by placing the bottom tip of the tube in liquid nitrogen until it starts to fizzle, then lower the tube into the liquid nitrogen at about 1mm/sec. This will allow for sample expansion upwards during freezing, thus preventing the tube from cracking. Rubber septa and commercial blue caps should be removed because they will admit liquid nitrogen, resulting in tube explosion and injury upon removal from storage. After freezing is complete, please check the tube to make sure that it’s not cracked. A damaged tube may burst or fall apart upon even slight warming and injure lab personnel or damage our EPR spectrometers.

Glassing Agents

Pure Substance		
3-methylpentane	sulfuric acid	sugar (.4 M sucrose)
methylcyclopentane	phosphoric acid	triethanolamine
paraffin oil (Nujol)	ethanol	2-methyltetrahydrofuan
isopentane	isopropanol	di-n-propyl ether
methylcyclohexane	1-propanol	decalin
isooctane	1-butanol	triacetin
boric acid	glycerol	toluene

Mixtures	
Components	*Ratio A:B:C...*
hydrocarbon	
3-methylpentane/isopentane	1:1
isopentane/methylcyclohexane	1:6
methylcyclopentane/methylcyclohexane	1:1
3-methylpentane/isopentane	1:2
alcohol	
ethanol/methanol	4:1, 5:2, 1:9
isopropanol/isopentane	3:7
ethanol/ispopentane/diethyl ether	2:5:5
isopentane/n-butanol	7:3
isopentane/isopropanol	8:2
diethyl ether/isooctane/isopropanol (or ethanol)	3:3:1
diethyl ether/isopropanol (or ethanol)	3:1
diethyl ether/toluene/ethanol	2:1:1
butanol/diethyl ether	2:5
aromatic	
toluene/methylene chloride	1:1 or excess toluene
toluene/acetone	1:1 or excess toluene
toluene/EtOH or MeOH	1:1 or excess toluene
toluene/acetonitrile	1:1 or excess toluene
toluene/chloroform	1:1 or excess toluene
water	
water/propylene glycol	1:1
water/glycerol	4:1 to 1:4
water/(poly)ethylene glycol	4:1 to 1:4

*Adapted from: Drago, R. S. Physical methods for chemists; 2nd ed.; Saunders College Pub: Ft. Worth, 1992.

Tubes

A suggested option is cutting tubes to 10cm in height, a qualified glassblower/ quartz worker could seal the extra top portion for obtaining two EPR tubes per 25cm commercial tube. A convenient cutting tool is a Restek ceramic scoring wafer (cat. #20116). Multiple X-band and Q-band tubes fit easily in a 15 or 50mL Falcon tube for efficient storage. The Falcon tube has about three 5mm holes on the side at 4 and 8cm from bottom (6 total on side) and a few 2mm holes on the top cap. These are easily introduced with an old soldering iron in a fume hood.

Preferred

X-band Wilmad PQ-706 ID = 2.8mm OD = 3.8mm L = 100mm*

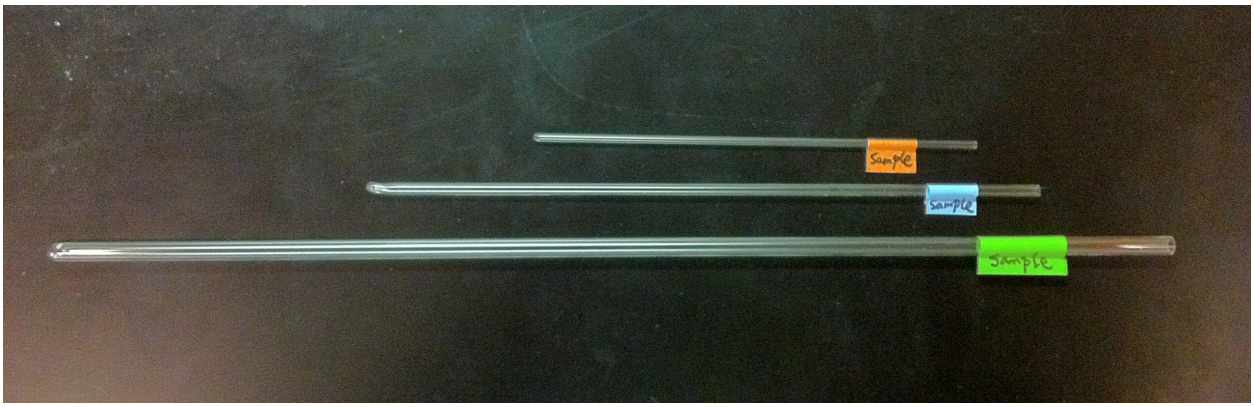
Q-band Vitrocom CV2024 ID = 2.0mm OD = 2.4mm L = 100mm

Minimum

X-band Wilmad SQ ID = 3mm OD = 4.0 ±0.1mm L = 100mm*

Q-band Vitrocom CV2024 ID = 2.0mm OD = 2.4mm L = 100mm

Labeling samples
When shipping multiple samples, it is important to label each tube properly. Do not use Scotch tape, as it does not stay on the tubes when frozen. Ideally, use hockey, fisher brand or medical type tape to make the labels. Use a permanent marker to label the tubes. Wrap the tape tightly around the tube and stick the end to itself, forming a “flag” with no more than a 5 mm length sticking out sideways. Also, it is good practice to use a grease pen to write an identifying number directly on the tube in case the label is lost. Please email us the sample information including concentration, solvents, individual labeling, as well as any specific care that needs to be taken with samples (e.g. temperature stability, oxygen sensitivity, light sensitivity).



Orange = Q-band, Blue = Ka-band, Green = X-band

Sample Concentration:
In most cases, protein concentration and EPR signal concentration (spins/ volume) are not equal. Iron-sulfur proteins are highly variable, often EPR of [4Fe-4S]+ signals are at 20-30% of protein concentration. An example is MoFe/holo-NifHDK protein of dinitrogenase, where samples are maximally concentrated up-to, but not including, formation of precipitates. This is ca. 100mg/mL for MoFe/ holo-NifHDK. Please use all methods readily available in your institution, such as UV-Vis and ICP-AES, to quantify the amount of the desired metal center in your enzyme prior to EPR analysis. If you have basic CW-EPR available in your department and any uncertainty remains, please test at least one sample to verify EPR spin concentration. Methods with a copper spin standard are outlined in W.R. Hagen, EPR spectroscopy as a probe of metal centres in biological systems, 2006 Dalton Transactions, pp. 4415–4434.

Preferred

400-2000 [(µmol spins)/L] of the desired signal;

DEER: 150 µM of bi-labeled protein, or 300 [(µmol spins)/L]. Max 250 bi-labeled protein or 500 [(µmol spins)/L].

Minimum

100 µM [(µmol spins)/L] of the desired signal – might limit ability to perform ENDOR and HYSCORE.

DEER: 100 µM of bi-labeled protein, or 200 [(µmol spins)/L].

Shipping frozen samples in EPR tubes
If you are shipping samples in a dry transport dewar, the dewar should have been filled with liquid N2 and allowed to sit overnight. Prior to shipping, make sure that the liquid nitrogen is completely drained (shippers will often be returned due to residual Liquid N_2). Fill the empty space near the samples with paper towels to secure the tubes in the dewar and prevent breakage.

Flame sealing tubes
For samples that are oxygen reactive, the tubes can be sealed under Ar, N_2, or just vacuum. If the sample is already in the tube, keep the bottom of the EPR tube in liquid N2 when sealing them at the top. (X Band tubes: SQ or PQ tubes: ID 3mm, OD ~ 4mm, respectively. Ka Band tubes (can be used at Q Band): ID 2mm, OD 2.4mm. Q Band tubes: ID 1.1mm, OD 1.6mm.) Remember to keep the diameter of the sealed tube smaller than the original diameter so we can load it into the spectrometer. Be careful with the open end of the tube, as it may be sharp.

Sample specific concerns
If you have other questions or concerns that are not addressed here, please contact us directly to address these specific issues before sending samples.

Shipping Address:

Department of Chemistry
University of California - Davis
One Shields Avenue
Davis, CA 95616