General guidelines for preparation of EPR samples for use at the Caltech EPR facility

Sample Tubes

Clear Fused Quartz (CFQ) tubes should always be used. Wilmad-LabGlass is a good source for purpose-made EPR tubes (Website: https://www.wilmad-labglass.com/ProductList.aspx?t=110).

For experiments performed on the continuous wave EPR spectrometer in B160, the standard quality (SQ) EPR tubes often sold in stockrooms are suitable. However, for experiments where an accurate quantitation of the paramagnetic species is desired, the more consistent PQ tubes from Wilmad are recommended.

As for samples intended for use in the pulse EPR spectrometer in B264, these tubes are not ideal for X-band experiments as they are much longer than is necessary, and the 25 cm length does not fit well into the sample rods used for the pulse EPR probes – tubes should be cut to 20 cm or shorter. Additionally, the economy-grade EPR tubes often sold in sold in chemistry stock rooms have fairly low manufacturing tolerances for the outer diameter (OD) – they can vary by about ± 0.2 mm. The probe used for X-band electron-nuclear double resonance (ENDOR) experiments (MD-4) has a maximum sample diameter of 4 mm, which many of these economy tubes exceed. For this reason, any X-band samples that are to be used in this probe must be either the (admittedly more expensive) precision quality (PQ) tubes of 4 mm or less, or have been confirmed to be 4.00 mm OD or smaller by carefully measuring using a high-precision caliper.

For Q-band (34 GHz) experiments, smaller diameter EPR tubes must be used – specifically a maximum of 1.8 mm OD. The preferred tubes with part numbers from Wilmad are shown below.

Preferred tubes:

X-band:	ELEXSYS Pulse/CW Instrument:	Wilmad PQ-714-100M ID = 2.8 mm OD = 4 mm L = 10 cm
	EMX CW Instrument:	Wilmad SQ-707-250M ID = 3 mm OD = 4 mm L = 25 cm
Q-band: (ELEXSYS only):		Wilmad WG-221T-RB ID = 1.1 mm OD = 1.6 mm L = 10 cm

Sample preparation and freezing

For a series of sample preparations which are to be compared directly, all tubes should be filled to the same volume. Sample heights are from the top of the sample to the beginning of 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 absolutely necessary. Make sure that there are no air bubbles and that sample is homogenous.

Preferred

X-band 15 mm sample height (20 mm for EMX X-band CW instrument in Crellin B160)
Q-band 10 mm sample height

Minimum

X-band 6 mm sample height
Q-band 5 mm sample height

Glassing Agents for frozen samples

Because EPR spectroscopy is often done on frozen samples at cryogenic temperatures, appropriate solvent systems that tend to form glass-like solids when frozen to ensure that paramagnetic analytes are randomly oriented and well separated. Please note that this is not an exhaustive list of all possible glassing solvent systems, but is meant as a general reference.

Pure Substance		
3-methylpentane	sulfuric acid	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
other organics	
acetonitrile / methylene chloride	1:1
water	
water / propylene glycol	1:1
water / polyethylene glycol	5:1 to 1:4
water / sucrose	~0.4 M sucrose
Water / glycerol	10:1 to 1:1

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

It is also feasible to use powders of solids, though pure compounds should typically should be diluted in a suitable salt (KBr for example).

Freezing of samples should be accomplished by placing the bottom tip (1-2 mm) of the tube in liquid nitrogen until the evaporated nitrogen gas layer dissipates, then lowering the tube slowly into the liquid nitrogen at about 1mm/sec. This will allow for sample expansion upwards during freezing, thus preventing the tube from cracking. Care should be taken to freeze the sample slowly enough that the sample freezes from the bottom up – rapidity of freezing will obviously differ between different solvents.

If stored in liquid nitrogen, 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 has not cracked. A damaged tube may burst or fall apart upon even slight warming and injure lab personnel or damage our EPR spectrometers.

Flame sealing tubes

For samples that are oxygen reactive, the tubes can be sealed under inert gas (Ar, N_2), or vacuum. If the sample is already in the tube, keep the bottom of the EPR tube in liquid N_2 when sealing them at the top. (X Band tubes: SQ or PQ tubes: ID 3mm, OD ~ 4mm, respectively 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.

Labeling 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 Fisher/VWR lab 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.

Sample Concentration

While the optimal concentration of the paramagnetic species of interest will depend on the nature of the EPR signal itself, the following general guidelines should apply to most EPR active species. In general the higher the better, but if the concentration is extremely high signals may become artificially broadened due to dipolar magnetic interactions between adjacent spin-active compounds.

<u>Preferred</u>

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

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

<u>Minimum</u>

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

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

Sample specific concerns

If you have other questions or concerns that are not addressed here, please contact me at directly phoyala@caltech.edu to address these specific issues before preparing samples.