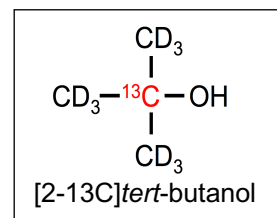


Studies to date have used perdeuterated [2-¹³C]*tert*-butanol as shown, purchased from Sigma Aldrich or Cambridge Isotopes.



For polarizing on **Hypersense** at 3.35T and 1.4K, the following preparation yields polarization levels competitive with [1-¹³C]pyruvate:

1. Combine *t*-butanol and high purity water (e.g. sterile reverse-osmosis filtered water at 17MΩ.cm or better) in the ratio 60% water: 40% *t*-butanol by volume.
2. To this mixture, add 15mM OX063 or AH111501 radical, and, if possible, 1.0mM Ho-DOTA (see below).
3. To ensure glassing, add sucrose equal to 41% of the weight of the water/butanol/radical mixture.

There are no special considerations for polarizing this mixture; simply run a microwave sweep to set the microwave frequency and polarize as with pyruvate. The buildup time constant is typically ~2500s, so three hours may be required to reach full polarization.

For *in vivo* studies in rodents, 250mg of the above mixture can be polarized and dissolved in 4ml of dissolution media. In our experience, higher liquid-state polarization is achieved with larger samples and larger volume of dissolution media. Typically, 525mg of sample dissolved in 10ml of media works well.

Normal saline containing 250mg/liter EDTA and buffered to neutral pH with 10mM TRIS and HCl is a suitable dissolution medium.

Holmium DOTA can be prepared as described in <https://pmc.ncbi.nlm.nih.gov/articles/PMC5199769/>

Like Gadolinium, Holmium yields modest improvements in polarization. However, unlike Gadolinium, Holmium does not appreciably shorten T₁ in the liquid state.

For polarization on **SpinAligner** at 6.7T, the radical concentration should be increased to 21.5mM. Other parameters have not yet been optimized.