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RESEARCH PRODUCTS

Instructions for Use

Ethyl 2-hydroxy-2-methyl-3-oxobutanoate (4-^{13}C, 99%; 2-methyl-D_3, 98%)

CDLM-10508

Catalog Numbers	Required Reagents	Required Equipment
CDLM-10508-0.25 CDLM-10508-1	0.06 M K_2HPO_4 (in H_2O) 10 N NaOH 6 N HCl D_2O (optional)	Small stir bar Magnetic stirrer Micro-pipette Micro pH electrode and pH meter Clamp

Suggested Hydrolysis Procedure for CDLM-10508-0.25:

1. Add 1.02 mL of 0.06 M K_2HPO_4 (in H_2O) to the vial. Tightly close the vial. Remove label to allow visualization of the vial's contents.
2. Mix contents in vial by vortexing for a minimum of 5 seconds. Ensure the entire inner surface of the vial and cap get thoroughly wetted.
3. Add small stir bar to vial. Clamp in place over magnetic stirrer to ensure contents to do not spill. Carefully insert calibrated pH electrode. Additional K_2HPO_4 (up to 1 mL) may be added to ensure adequate coverage of the electrode.
   Add 50 µL of D_2O to serve as a signal for the lock (optional).
4. While stirring, carefully add NaOH in 10-20 µL amounts until pH reaches 11-12. DO NOT exceed pH 13. The pH will decrease as the hydrolysis reaction proceeds. Add additional NaOH once the pH drops below ~9.5.
5. Once the pH stabilizes, remove aliquot to measure ^{1}H-NMR spectrum.
   a. The extent of the hydrolysis reaction is monitored by either inspection of the $^{13}CH_3$ resonance or the triplet and/or quartet from the ethyl group. The chemical shifts for the $^{13}CH_3$ doublet and the methyl group on the ethyl group each shift upfield by ~0.1 ppm after hydrolysis. At a pH of ~11, the methylene proton from the ethyl group resonates at ~4.1 ppm while in the ester form and resonates at ~3.5 ppm after hydrolysis.
   b. Proceed to the next step only if the hydrolysis reaction is complete. If the hydrolysis reaction is incomplete, transfer contents from NMR tube to the original vial and add additional NaOH base and wait until the pH stabilizes.
6. Once verified by ^{1}H-NMR that the hydrolysis reaction is complete, add contents of NMR tube back to vial. While stirring, carefully add 6 N HCl to bring pH to 7.4.
7. Transfer contents to 1 L of suitable labeled minimal media 1 hr before induction.

Suggested Hydrolysis Procedure for CDLM-10508-1:

1. Add 4.08 mL of 0.06 M K_2HPO_4 (in H_2O) to the vial. Tightly close the vial.
2. Follow steps 2-6 stated above for CDLM-10508-0.25.
3. Divide solution into four equal aliquots, then freeze at -80°C or use fresh. Each aliquot contains enough precursor to prepare 1 L of suitable labeled minimal media.

For technical assistance, please contact Dr. Kevin Millis, Senior Scientist, Applications Development, at 1.978.749.8000 x1943 or kmillis@isotope.com.