

Neutral Lipid Internal Standard
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This protocol is for the preparation of the deuterated internal standard solution for the neutral loss screening of diacyl- and triacylglycerol species from cells.

Reagents required:

Toluene

Methanol

Individual internal standards (listed below) at 1 mg/mL in CH₂Cl₂ (add 1mL of solvent to the original vial containing 1 mg of each standard; store at -20 °C in vials with Teflon-lined caps)

30 mL amber glass bottle (Schott Type I plus, obtained from Avanti Polar Lipids)

d5-Standard	μL for 20 μmol	<i>m/z</i>		Avanti ID/LM ID
		[M+H]⁺	[M+NH₄]⁺	
14:0/14:0 DAG	10.36	518.8	535.8	110535
15:0/15:0 DAG	10.92	546.9	563.9	110536
16:0/16:0 DAG	11.48	574.9	591.9	110537
17:0/17:0 DAG	12.04	602.9	619.9	110538
19:0/19:0 DAG	13.16	658.9	675.9	110539
20:0/20:0 DAG	13.72	686.9	703.9	110540
14:0/16:1/14:0 TAG	15.07	754.7	771.7	LMGL03010009
15:0/18:1/15:0 TAG	16.20	810.8	827.8	LMGL03010010
16:0/18:0/16:0 TAG	16.80	840.8	857.8	LMGL03010011
17:0/17:1/17:0 TAG	17.04	852.8	869.8	LMGL03010012
19:0/12:0/19:0 TAG	16.80	840.8	857.8	LMGL03010013
20:0/20:1/20:0 TAG	19.56	978.9	995.9	LMGL03010014
20:2/18:3/20:2 TAG	18.75	938.5	955.5	LMGL03010015
20:4/18:2/20:4 TAG	18.64	932.8	949.8	LMGL03010016
20:5/22:6/20:5 TAG	19.51	976.5	993.5	LMGL03010008

1. Add volume given for each standard in table to the specified bottle.
2. Dry the mixture under nitrogen.
3. Add 20 mL of Toluene/MeOH (1:1).
4. Use the LIMS "Solution" application for assignment of a barcode ID for this solution.
5. Store at -20 °C.
6. Add 100 μL to each time course sample immediately after cells are harvested.

