

LSC in Practice

Tritium Substrates on Silica TLC — Catecholamine Assays

Problem

The manager of one of PerkinElmer’s application support departments requested assistance for a researcher who was performing catecholamine assays. After sample preparation with ULTIMA Gold™ (PerkinElmer part number 6013329), the researcher had been experiencing high backgrounds.

Discussion

According to the submitted request, after completion of the enzyme reaction, the sample was extracted and run on silica TLC plates. Subsequently, the plate was scraped, and then treated to release the sample. The final steps, prior to counting, are the addition of 1 mL of 0.05 N ammonium hydroxide to the silica, then the addition of 50 µL of 4% sodium periodate (NaIO₄) and 50 µL of 10% glycerol. Finally, 7 mL of ULTIMA Gold are added and the sample is counted using LSA at ambient temperature using plastic vials.

Upon receipt of this request, we checked the referenced lot of ULTIMA Gold with each of the sample’s constituents to determine if there was any contribution to background from these combinations. The results were as follows:

Background Contribution in 7.0 mL ULTIMA Gold		
Sample	CPM After 2 Minutes (0-18.6 keV)	CPM After 10 Minutes (0-18.6 keV)
1.0 mL 0.05 M ammonia	18	13
50 µL 4% NaIO ₄	15	15
50 µL 10% glycerol	15	14
Combined constituents	15	14

These results show that the reagents, either singly or in combination, do not produce high backgrounds in ULTIMA Gold.

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We noticed the comment that the researcher had found “really high counts in background samples” and this was the clue which led us to the cause of the problem.

Basically, the high backgrounds were coming from the TLC plates, or more correctly, from the fluorescence indicator that was present in the silica.

To demonstrate the effect, we prepared various samples in ULTIMA Gold and checked the backgrounds. The results are shown below:

Backgrounds in 7.0 mL ULTIMA Gold	
Sample	CPM in 0-18.6 keV After 2 Minutes
ULTIMA Gold	15
ULTIMA Gold + silica (no indicator)	16
ULTIMA Gold + silica (with indicator)	100
ULTIMA Gold + silica (no indicator) + 1.0 mL 0.05 M NH ₃	16
ULTIMA Gold + silica (with indicator) + 1.0 mL 0.05 M NH ₃	35,000

From the results it can be seen that the fluorescence indicator can be rapidly extracted by 0.05 M ammonia and not by the ULTIMA Gold. The 100 CPM recorded for the ULTIMA Gold + silica (with indicator) rapidly decayed to normal background levels within 10 minutes. Despite the return to normal backgrounds, it should be remembered that ULTIMA Gold is an emulsifying cocktail that contains detergents. Therefore, over time, ULTIMA Gold will have some capacity for extracting the fluorescence indicator leading to uncertain results if the samples are retained and recounted.

Recommendation

The problem the researcher has experienced can be avoided by obtaining silica that does not contain a fluorescent indicator.



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