

LSC in Practice

Quenching Effects of Chloroform

Problem

Our field representative asked for application assistance for a researcher who wished to improve the ³H efficiency of a PerkinElmer FLO-ONE™ A-525 flow scintillation analyzer with a sample that contained chloroform. In general, the researcher had been experiencing poor results.

The researcher had attempted to improve counting results by using several of PerkinElmer's safer ULTIMA-Flo™ and classical Flo-Scint™ cocktails without better results. At present, the laboratory was achieving reasonable results (³H eff. 25% to 30%) by running the experiment in a conventional LSC counter in 20 mL polyethylene vials with PerkinElmer's ULTIMA Gold™ cocktail (PerkinElmer part number 6013329).

The researcher wanted help to improve the efficiency of his results.

Discussion

The researcher's sample was composed of 20.0 mL chloroform, 9.0 mL methanol and 1.0 mL water.

We tested the system using the specified chloroform/methanol/water mixture. Our tests were performed with ULTIMA Gold in polyethylene vials by adding 1.0 mL of the sample to 10.0 mL ULTIMA Gold.

We also varied the procedure to help determine which conditions might lead to a more optimal counting situation.

The variations included:

1. Increasing the volume of cocktail.
2. Changing from chloroform/methanol/water to dichloromethane/methanol/water. This change may be of benefit since dichloromethane (two chlorine atoms, i.e., CH₂Cl₂) is less quenching than chloroform (three chlorine atoms, i.e., CHCl₃).

We measured the ³H efficiency of ULTIMA Gold (10 mL and 15 mL) and ULTIMA-Flo M (10 mL and 15 mL) (PerkinElmer part number 6013579), then added 1 mL of the different mixtures, since these are the main quenchers, and measured the ³H efficiency of the total system. The results are shown below:

Cocktail	No Sample	+Chloroform/MeOH/H ₂ O
ULTIMA Gold (10 mL)	58.5%	38.4%
ULTIMA Gold (15 mL)	58.5%	43.7%
ULTIMA-Flo M (10 mL)	47.5%	13.5%
ULTIMA-Flo M (15 mL)	47.5%	20.2%

Cocktail	No Sample	+Dichloromethane/MeOH/H ₂ O
ULTIMA Gold (10 mL)	58.5%	48.2%
ULTIMA Gold (15 mL)	58.5%	52.3%
ULTIMA-Flo M (10 mL)	47.5%	31.5%
ULTIMA-Flo M (15 mL)	47.5%	36.0%

These results indicate the following:

Increasing the volume of cocktail from 10 mL to 15 mL increases the efficiency by:

- Approximately 5% for ULTIMA Gold with added chloroform/MeOH/H₂O [i.e., 38.4% up to 43.7%].
- Approximately 7% for ULTIMA-Flo M with added chloroform/MeOH/H₂O [i.e., 13.5% up to 20.2%].

In addition, when dichloromethane is used instead of chloroform the efficiency increase is:

- 10% for ULTIMA Gold (10 mL) [i.e., 38.4% up to 48.2%].
- 8.5% for ULTIMA Gold (15 mL) [i.e., 43.7% up to 52.3%].
- 18% for ULTIMA-Flo M (10 mL) [i.e., 13.5% up to 31.5%].
- 16% for ULTIMA-Flo M (15 mL) [i.e., 20.2% up to 36.0%].

Recommendations

After this review, we recommended changing from the highly quenching chloroform to less quenching dichloromethane. In addition, if the researcher can adjust the ratio of cocktail to eluent such that there is additional cocktail, the counting efficiency would be increased. The biggest benefit will be seen by changing from chloroform to dichloromethane.

Follow-Up Comment

We received a fax from this researcher which confirmed that the chloroform has been replaced with dichloromethane and the chromatogram was not altered. Furthermore, a larger volume FSA cell (5 mL) and mixing ratio of 1:5 (Sample:ULTIMA-Flo M) was being used. The efficiency had increased to 18% to 19% allowing the laboratory to run the experiment using the FLO-ONE without fraction collection, as they had hoped.