

LSC in Practice

Overcoming Chemical and Color Quench with Bile Acid Samples

Problem

A researcher observed a count rate that was lower than expected with a tSIE of 300-400. The sample was bile acid that had been recovered from mouse feces. Water was used to rehydrate the feces. An equal volume of 2:1 chloroform:methanol was added and this was centrifuged. The bottom fraction was recovered and the extraction was repeated. The final sample volume was between 15-25 mL. A 0.5 mL aliquot of this sample was counted in 10 mL of cocktail. The researcher recognized the need to reduce the effects of chemical and color (yellow) quench and had tried H₂O₂ to decolorize the sample without success. The researcher wanted to know if bleach was a good alternative.

Discussion

Since this is a true sample preparation problem, the exact cocktail chosen for counting has not been considered for this problem. The experiment was suffering from a combination of both chemical and color quench. With conventional bile, the color effect is particularly difficult to remove. However, in feces the amount of bile acid is considerably diminished and most of the color present would be due to the feces.

The water is merely used to rehydrate the feces prior to solvent extraction with chloroform:methanol.

Our testing showed that the chloroform:methanol was extracting color from the feces. Consequently, we considered the following potential alternatives:

1. Feces solubilization (which would only be suitable if no other radioactive contaminant was present). This method will eliminate all, or a majority, of the color quench problem while considerably reducing the chemical quench.
 - a) Weigh 100-200 mg of fecal homogenate (wet weight) into a 20 mL glass vial.
 - b) Add 0.2 mL of 70% (v/v) perchloric acid and 0.4 mL of 100 volume hydrogen peroxide.
 - c) Tightly cap the vial and place in an oven at 75 °C for 1-1/2 to 2 hours.
 - d) Remove the vial and cool in an ice/water bath for 15 minutes.
 - e) Remove the cap and rapidly add 0.5 mL of 0.1 N sodium hydroxide. Replace the cap and shake.
 - f) Let stand for 30 minutes to allow absorption of any CO₂ by the sodium hydroxide solution.
 - g) Add 15 mL of Hionic-Fluor™ (part number 6013319) and count.
2. Pretreatments

Color in the sample could be removed, or bleached, prior to the extraction step. To accomplish this, the researcher could try either hydrogen peroxide or bleach prior to the extraction step.

Our recommended safer cocktail is PerkinElmer ULTIMA Gold™ XR, with Pico-Fluor™ 40 the classical cocktail alternative. Both cocktails will perform well; however, we always consider laboratory safety and environmental safety key points to consider when recommending any of our products.
3. Effect of chloroform

Chloroform is a well known quenching agent. Undoubtedly the presence of chloroform in the sample is the single largest factor affecting the quench. The replacement or removal of chloroform in this system should be considered.

One suggestion is to use an alternative mixture such as xylene, a toluene:methanol mixture or some other less quenching species. The second suggestion is to evaporate the final combined extracts to a low volume (thus removing most of the chloroform) and then reconstitute to the

original volume with an alternative less quenching solvent in which the bile acids are soluble. Please remember that chloroform is one of the strongest chemical quenchers and virtually any other solvent is preferable.

Recommendation

Our recommendation, in this case, is to perform solubilization of the feces, as outlined in alternative one, on the previous page. This alternative will provide a simple approach towards overcoming the problem.

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