

Pico-Fluor™ MI for Counting Biological Samples by Direct Addition

By Jock Thomson, Packard BioScience Company, Meriden, Connecticut

Introduction

Certain biological samples such as milk, urine, serum, and plasma have always presented researchers with difficulties when attempting to count these sample types directly in an LSC cocktail^{1,2}. Due to the complex nature of these samples, it has always been either difficult or almost impossible to incorporate them directly into a cocktail. Consequently, researchers have had to revert to time-consuming and laborious sample preparation methods such as distillation for urine and freeze drying for milk. In a recent situation, we were requested to provide a cocktail that would perform with plasma, serum, urine, and milk samples originating from rats. This challenging array of samples led to the development of a custom cocktail, which is designated Pico-Fluor™ MI. The performance of this new cocktail with these difficult biological sample types is so impressive that Pico-Fluor MI has been added to the already impressive range of LSC cocktails. Pico-Fluor MI, like all other Pico-Fluor cocktails, is based on pseudocumene solvent and has a flash point of approximately 49 °C.

Performance

Other LSC cocktails are unable to accept some or all of these samples, due to either compatibility problems or phase separation problems upon standing. These problems are not evident with Pico-Fluor MI. A summary of performance with various biological sample types is shown in Table 1.

Milk

What is milk?

Milk is an oil-in-water type of emulsion, stabilized by complex phospholipids and proteins bound to the surface of the fat globules. It contains proteins in colloidal dispersion, lactose in true solution, minerals, fat-soluble and water-soluble vitamins, enzymes, and other organic compounds. Milk's white appearance is produced by two salts, calcium caseinate and calcium phosphate. The key components presented in milk are water, fat, protein, lactose, minerals and other vitamins. Water is typically the major component (84-90%). The fat present is a complex mixture of lipid and triglycerides and is extremely variable between and within species e.g., human and cow milk contains 2-6% fat while seal milk is 60% fat. The major milk proteins are casein and the whey proteins called lactalbumin and lactoglobulin. Lactose, a disaccharide composed of glucose and galactose, is the major carbohydrate found in milk. The mineral ash (<1%) contains calcium, phosphorus, magnesium, potassium, sodium, chlorine, sulfur, and others in trace amounts.

Table 1.

Performance of Various LSC Cocktails with Biological and Other Samples.

	<i>mL sample per 10.0 mL cocktail</i>			
	Pico-Fluor MI	Ultima Gold™	Hionic-Fluor™	Pico-Fluor 40
Milk (cows)	10.0 mL	0.5 mL	1.5 mL	2.5 mL
Plasma (human)	2.0 mL	1.0 mL*	—	—
Serum (calf)	2.0 mL	1.0 mL*	0.5 mL*	0.5 mL*
Urine (human)	4.5 mL	8.0 mL*	1.5 mL	3.0 mL
Water	2.7 mL	3.2 mL	1.2 mL	2.3 mL
0.01 M PBS	6.2 mL	6.5 mL	1.4 mL	10.0 mL
³ H Efficiency (unquenched)	~52%	~55%	~50%	~51%

*Turns hazy on standing.

What LSC cocktails are suitable?

Currently the preparation of milk for LSC counting involves freeze drying which can take up to eight hours to generate a sample suitable for adding to a cocktail. Pico-Fluor MI can accept up to 10 mL of milk per 10 mL cocktail, thus opening up the possibility of rapidly screening multiple samples for isotopes such as ³H and ¹⁴C.

Table 2 illustrates the applicability of Pico-Fluor MI for milk and shows the limitation of other LSC cocktails.

Plasma & Serum

What are Plasma and Serum?

Plasma is the liquid portion of the blood; a protein-salt solution in which red and white blood cells and platelets are suspended. Plasma, which is 90 percent water, constitutes 55 percent of blood volume. Plasma contains albumin (the chief protein constituent), fibrinogen (responsible, in part, for the clotting of blood) and globulins (including antibodies). Plasma is obtained by separating the liquid portion of blood from the cells.

Blood serum is the clear liquid that separates from the blood when it is allowed to clot completely. It is, therefore, blood plasma from which fibrinogen has been removed in the process of clotting.

What LSC cocktails are suitable?

In general, direct sample addition is only recommended for small sample volumes (up to 1 mL) due to sample/cocktail incompatibility and color quench problems. The sample/cocktail incompatibility, which is marked by the appearance of a wispy precipitate, is principally due to the presence of proteinaceous material in the plasma and serum. The appearance of this protein precipitate is significantly influenced by the surfactants present in the LSC cocktail and certain surfactants can either accelerate or suppress its appearance. In addition, certain alcohols such as ethanol and isopropyl alcohol are particularly effective in suppressing the appearance of the precipitate. Pico-Fluor MI, due to its optimized blend of surfactants is capable of accepting up to 1 mL of plasma or serum and remains clear for 48 hours. Ultima Gold and Ultima-Flo™ M are also suitable, but with Ultima-Flo M lower ³H efficiency is obtained. To improve stability with Ultima Gold and Ultima-Flo M, the addition of up to 1 mL of either ethanol or isopropyl alcohol per 10 mL cocktail suppresses the appearance of the protein precipitate. With difficult samples such as plasma and serum from smaller animals, it may not be possible to completely suppress the appearance of the protein precipitate. In this case the only solution is to complete counting within 24 hours of sample preparation.

Table 2.
Performance of Various LSC Cocktails with Milk.

Cocktail	Type	Max. capacity at 20 °C*	Comments
Pico-Fluor MI	Classical	10.0 mL	
Ultima Gold	Safer	0.5 mL	Above 0.5 mL sample haziness occurs after 1 hour.
Ultima Gold XR	Safer	No capacity	
Ultima Gold AB	Safer	No capacity	
Ultima Gold LLT	Safer	No capacity	
Opti-Fluor®	Safer	4.5 mL	Above 3.0 mL sample a liquid crystal effect is seen.
Emulsifier Safe™	Safer	1.0 mL	
Hionic-Fluor	Classical	1.5 mL	
Insta-Gel® Plus	Classical	2.0 mL	
Pico-Fluor 40	Classical	2.5 mL	

*Capacities are per 10.0 mL cocktails and cows milk was used throughout.

Urine

What is Urine?

Urine is approximately 95% water, and it usually contains sodium chloride, urea, uric acid, and creatinine. It may contain a trace of amino acids and varying amounts of electrolytes, depending upon dietary intake. Urea is a by-product of amino acid metabolism and uric acid results from the metabolism of nucleic acids. Refrigeration of the urine specimen is necessary to help preserve the urine composition and specimen integrity.

What LSC cocktails are suitable?

Direct sample addition is possible for any sample volume as there are LSC cocktails available which can accept up to 1:1 ratio of urine in the cocktail. However, color quench problems will be encountered at such a high sample loading. In general, sample volume is restricted to no more than 3.0 mL per 10 mL LSC cocktail to minimize the effect of color quench, especially if ^3H is the isotope of interest. Pico-Fluor MI is particularly good with urine, as it is capable of inhibiting the protein precipitation also found with this particular sample type. Other suitable cocktails include Ultima-Gold

LLT that has been successfully used where sample sizes are kept low. As mentioned earlier some researchers have distilled the urine sample, which overcomes the sample/cocktail incompatibility, and the color quench problems, but the drawback is that this increases sample preparation time considerably. A variety of cocktails are capable of accepting urine samples and these are summarized in Table 3.

Conclusion

The data shows that Pico-Fluor MI is ideally suited for handling these biological sample types where direct addition to a cocktail is the preferred sample preparation technique. There are a variety of LSC cocktails of both safer, high flash point and classical solvent types that are suitable for use in counting biological samples. In addition, there are a variety of reagents that can be used to prepare the samples for LSC. If problems with sample preparation or cocktail selection persist or help is needed with a particular sample type not mentioned in this publication, please call your local PerkinElmer representative for further applications support.

Table 3.
Recommended LSC Cocktails and Capacities for Urine.

Cocktail	Type	Max. capacity at 20 °C*	Comments
Pico-Fluor MI	Classical	4.0 mL	Remains clear up to 4.0 mL sample for >24 hours.
Ultima Gold	Safer	8.0 mL	Slight wispy precipitate appears after 24 hours at >2.0 mL sample.
Ultima Gold XR	Safer	10.0 mL	Slight wispy precipitate appears after 24 hours at >3.0 mL sample.
Ultima Gold LLT	Safer	10.0 mL	Slight wispy precipitate appears after 24 hours at >2.0 mL sample.
Ultima Gold AB	Safer	10.0 mL	Slight wispy precipitate appears after 24 hours at >2.0 mL sample.
Opti-Fluor	Safer	9.0 mL	Slight wispy precipitate appears after 24 hours at >3.0 mL sample.
Hionic-Fluor	Classical	1.5 mL	>2.0 mL sample forms a gel.
Pico-Fluor 40	Classical	3.0 mL	Slight wispy precipitate appears after 24 hours at >3.0 mL sample.

*Capacities are per 10.0 mL cocktails and human urine was used throughout.

References

1. Kobayashi, Y. and Maudsley, D.V., Biological Applications of Liquid Scintillation Counting, 153-159.
2. Application Note AN9002-CSR (1999), *Sample Preparation and Counting of Biological Samples*. PerkinElmer.

Supplementary information

Pico-Fluor MI (PerkinElmer PN# 6013139.....2 x 5Lt)

Classical (pseudocumene based) cocktail tailored for use with biological samples such as plasma, serum, milk and urine. Originally designed for use with biological samples originating from rats and goats.

Ultima Gold (PerkinElmer PN# 6013329.....2 x 5Lt)

Safer, biodegradable, high flash point cocktail (di-isopropylnaphthalene based), which can accept a wide variety of aqueous sample types.

Opti-Fluor (PerkinElmer PN# 6013199.....2 x 5Lt)

Safer, biodegradable, high flash point cocktail (linear alkylbenzene based), designed for use with dilute aqueous sample types at sample volumes up to 2 mL in 10 mL cocktail.

Hionic-Fluor (PerkinElmer PN# 6013319.....2 x 5Lt)

Classical (pseudocumene based) cocktail tailored for use with solubilized samples. Exhibits extremely fast chemiluminescence decay and is therefore, suitable for use with Soluene[®]-350 and SOLVABLE™.

Pico-Fluor 40 (PerkinElmer PN# 6013349)

Classical (pseudocumene based) cocktail originally designed to accept high sample volumes. Exhibits a high resistance to chemiluminescence and also is suitable for use with Soluene-350 and SOLVABLE.