LSC sample preparation and counting of biological samples.

Highlights

- Use of solubilization for biological samples.
- Recommended cocktail choices for counting biological samples.
- Specific sample preparation techniques for various biological samples.

Introduction

In liquid scintillation counting (LSC), preparation of biological samples has always been treated as a special case because of the difficulties associated with their preparation for counting. In general, all biological samples contain components not generally found in any other sample types, therefore it is of benefit to understand the composition of these samples to fully understand the need for the slightly unusual sample preparation methods.

Sample types

1. Whole blood

Whole blood is living tissue circulating through the heart, arteries, veins, and capillaries carrying nourishment, electrolytes, hormones, vitamins, antibodies, heat, and oxygen to the body's tissues. Whole blood contains red blood cells, white blood cells and platelets suspended in a watery fluid called plasma.

Suggested methods:

The successful preparation of blood samples[1] for LSC can often be technically difficult, and successful digestion can be largely dependent on the practical experience of the researcher.

The source of blood and the correct choice of solubilizer also influence the results of digestion. In general, most of the sample preparation problems occur with blood samples from smaller animals such as rats and mice. In this case, it may be necessary to consider smaller sample volumes, and even then, the end result is usually color quench problems. Suggested sample preparation methods include solubilization and sample combustion. Direct sample addition is not recommended due to color quench and sample/cocktail incompatibility.

Solubilization methods are given below for both Soluene®-350 and SOLVABLE™, Revvity's well-referenced solubilizers[3], and the final choice of method rests with the individual researcher.

Soluene-350 method

1. Add a maximum of 0.4 mL of blood to a glass scintillation vial.
2. Add, while swirling gently, 1.0 mL of a mixture of Soluene-350 and Isopropyl Alcohol (IPA) at 1:1 or 1:2 ratio. Ethanol may be substituted for the IPA if desired.
3. Incubate at 60 °C for 2 hours. The sample at this stage will be reddish-brown.
4. Cool to room temperature.
5. Add 0.2 mL to 0.5 mL of 30% hydrogen peroxide dropwise or in small aliquots. Foaming will occur after each addition, therefore, gentle agitation is necessary. Keep swirling the mixture until all foaming subsides and then continue swirling until all of the hydrogen peroxide has been added. Hydrogen peroxide treatment helps reduce the amount of color present and thus reduces color quench in the final mixture.
6. Allow to stand for 15 to 30 minutes at room temperature to complete the reaction.
7. Cap the vial tightly and place in an oven or water bath at 60 °C for 30 minutes. The samples at this stage should now have changed to pale yellow.
8. Cool to room temperature and add 10 to 15 mL of either Hionic-Fluor™, Pico-Fluor Plus or Ultima Gold™. If color is present use 15 mL cocktail, as this reduces color quench by diluting the color.
9. Temperature and light adapt for 1 hour before counting.

SOLVABLE method

1. Add a maximum of 0.5 mL blood to a glass. scintillation vial.
2. Add 1.0 mL SOLVABLE.
3. Incubate the sample at 55 – 60 °C for 1 hour. Sample at this stage will be brown/green in appearance.
4. Add 0.1 mL of 0.1 M EDTA-di-sodium salt solution which helps reduce foaming when the subsequent hydrogen peroxide is added.
5. Add 0.3 mL to 0.5 mL of 30% hydrogen peroxide in 0.1 mL aliquots. Gently agitate between additions to allow reaction foaming to subside. Hydrogen peroxide treatment helps reduce the amount of color present, and thus reduces color quench in the final mixture.
6. Allow to stand for 15 to 30 minutes at room temperature to complete the reaction.
7. Cap the vial tightly and place in an oven or water bath at 55 – 60 °C for 1 hour. The color will change from brown/green to pale yellow.
8. Cool to room temperature and add 10 to 15 mL of either Ultima Gold, Opti-Fluor™, Hionic-Fluor or Pico-Fluor Plus. If color is present, use 15 mL cocktail, as this reduces color quench by diluting the color.
9. Temperature and light adapt for 1 hour before counting.

2. 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% water, constitutes 55% 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. Therefore, serum is blood plasma with the fibrinogen removed by the process of clotting.

Suggested methods:

Possible methods include direct sample addition, solubilization and sample combustion. 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 becomes apparent by the appearance of a wispy precipitate, is principally due to the presence of proteinaceous material, e.g. albumin, in the plasma. 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 IPA are particularly effective in suppressing the appearance of the precipitate.

General guidelines:

Direct addition

Up to 1 mL of plasma or serum can be added to certain cocktails to allow reproducible counting. For this method, Ultima Gold is recommended. In addition, Ultima-Flo™ M is also suitable, but its use results in lower ^{3}H efficiency. With these cocktails, it is possible to add up to 1 mL of either ethanol or IPA per 10 mL cocktail, to suppress 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.

Solubilization

1. For each 1.0 mL of plasma or serum add 1.0 mL of SOLVABLE or Soluene-350.
2. Incubate the sample at 55 – 60 °C for 1 hour.
3. Add 0.3 mL to 0.5 mL of 30% hydrogen peroxide in 0.1 mL aliquots. Gently agitate between additions to allow reaction foaming to subside. Hydrogen peroxide treatment helps reduce the amount of color present and thus reduces color quench in the final mixture.
4. Allow to stand for 15 to 30 minutes at room temperature to complete the reaction.
5. Cap the vial tightly and place in an oven or water bath at 55 – 60 °C for 1 hour.
6. Cool to room temperature and add 10 to 15 mL of appropriate cocktail (Ultima Gold, Opti-Fluor, Hionic-Fluor or Pico-Fluor Plus for SOLVABLE, and Hionic-Fluor, Pico-Fluor Plus or Ultima Gold for Soluene-350).
7. Temperature and light adapt for 1 hour before counting.

3. 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.

Suggested methods:

Possible methods include direct sample addition and sample combustion. 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 < 3.0 mL to minimize the effect of color quench, especially if ^{3}H is the isotope of interest.

As with plasma, there is a potential for sample/cocktail incompatibility, which becomes apparent by the appearance of a wispy precipitate and is principally due to the presence of proteinaceous material in the urine. 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 IPA are particularly effective in suppressing the appearance of the precipitate. In some cases, researchers have distilled the urine sample which overcomes the sample/cocktail incompatibility and the color quench problems. The only drawback is that this method increases sample preparation time considerably.

Direct addition

Cocktail selection dictates the sample loading for urine samples, but, as stated previously, sample volume is generally restricted to < 3.0 mL to minimize the effect of color quench. For this method, Ultima Gold (up to 8.0 mL in 10.0 mL cocktail) and Ultima Gold LLT

(up to 10.0 mL in 10.0 mL cocktail) are recommended. With all these cocktails, it is possible to add up to 1 mL of either ethanol or IPA per 10 mL cocktail to suppress the appearance of the protein precipitate. Where it is not possible to completely suppress the appearance of the protein precipitate, the only solution is to complete counting within 24 hours of sample preparation.

With difficult samples such as urine from smaller animals, there may be significant problems with sample/cocktail incompatibility. In this case, it may be necessary to either keep the urine sample volume below 0.5 mL per 10 mL cocktail or dilute the urine sample with an equal volume of deionized water. The capacities of cocktails for urine are shown in Table 1.

Table 1. Recommended LSC cocktails and capacities for urine.

Cocktail	Type	Max. capacity at 20 ˚C*	Comments
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 AB	Safer	10.0 mL	Slight wispy precipitate appears after 24 hours at >2.0 mL sample
Ultima Gold LLT	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
Emulsifier Safe™	Safer	2.5 mL	Slight wispy precipitate appears after 24 hours at >3.0 mL sample
Insta-Gel® Plus	Classical	1.0 mL	Slight wispy precipitate appears after 24 hours at >3.0 mL sample
Pico-Fluor Plus	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.			

4. Feces

Feces are body waste formed of undigested food that has passed through the gastrointestinal system to the colon. Feces are produced and stored in the colon until eliminated. A proportion of feces is inner gut lining. Normally, when blood cells become old, they are trapped and destroyed by the spleen. When this occurs, the hemaglobin must be broken down in the liver to bilirubin in order to be disposed of. Bilirubin is eventually excreted in the bile and leaves the body in the feces. Its presence is the reason why feces are brown in color.

Suggested methods:

The digestion of feces[2] strongly depends on the type of animal. It is possible to use both Soluene-350 and SOLVABLE, however, there can be problems with residual color and incomplete digestion due to the presence of cellulose-type material present in feces from species such as rat and rabbit. If Soluene-350 or SOLVABLE is to be used, then the method is as follows:

1. Add 0.1 mL water to 20 mg dried feces and rehydrate for 30 minutes.
2. Add 1.0 mL of SOLVABLE or Soluene-350.
3. Incubate the sample at 50 °C for 1 to 2 hours.
4. Add 1.0 mL IPA and mix. Stand for 2 hours at 50 °C.
5. Add 0.2 mL of 30% hydrogen peroxide dropwise and gently agitate. Hydrogen peroxide treatment helps reduce the amount of color present and thus reduces color quench in the final mixture.
6. Allow to stand for 15 to 30 minutes at room temperature to complete the reaction.
7. Cap the vial tightly and place in an oven or water bath at 50 °C for 1 hour.
8. Cool to room temperature and add 10 to 15 mL of appropriate cocktail (Ultima Gold, Opti-Fluor, Hionic-Fluor or Pico-Fluor Plus for SOLVABLE, and Hionic-Fluor, Pico-Fluor Plus or Ultima Gold for Soluene-350).
9. Temperature and light adapt for 1 hour before counting.

As an alternative, the use of a sodium hypochlorite solution is recommended. Sodium hypochlorite resolved a problem for one researcher (unpublished work) who was attempting to digest guinea pig feces. Sodium hypochlorite substantially digested this sample rapidly, but traces of undigested cellulose material remained. Isotope recoveries of greater than 98% for ^{3}H were achieved and this recovery level was confirmed by combustion in a Sample Oxidizer (Sample Oxidizer, Model 307). The solubilization method used for processing this feces sample is:

1. Weigh 50 to 150 mg of feces into a 20 mL glass scintillation vial.
2. Add 0.5 mL of sodium hypochlorite solution and cap tightly.
3. Heat in an oven or water bath at 50 – 55 °C for about 30 to 60 minutes with occasional swirling.
4. Cool to room temperature.
5. Remove the cap and blow out any remaining chlorine using a gentle stream of air or nitrogen.
6. Add 15 mL of Hionic-Fluor and shake to form a clear mixture.
7. Temperature and light adapt for 1 hour before counting.

5. 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 present 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 (~96%) and is extremely variable between and within species e.g. human and cow milk contains 2 – 6% fat, while seal milk is 60% fat. Proteins are present (3 – 4%), and several types are found in milk. The major milk proteins are casein (about 80%) and the whey proteins called lactalbumin and lactoglobulin. Lactose, a disaccharide composed of glucose and galactose, is the major carbohydrate found in milk (4 – 5%). The mineral ash (<1%) contains calcium, phosphorus, magnesium, potassium, sodium, chlorine, sulfur, and others in trace amounts. Whole milk contains some of all the vitamins known to be required by man.

Suggested methods:

The main isotopes of interest potentially present in milk, include ^{3}H, ^{14}C, ^{45}Ca, ^{89}Sr, ^{90}Sr, ^{131}I, ^{134}Cs and ^{137}Cs. Essentially, there are three methods suitable for handling milk samples, chemical separation after ashing, sample oxidation (combustion) and direct addition to cocktail. Chemical separation after ashing is necessary for measuring ^{45}Ca, ^{89}Sr, ^{90}Sr, ^{131}I, ^{134}Cs and ^{137}Cs, and there are numerous methods cited in the literature for such analyses. As mentioned previously, sample oxidation is only suitable for the determination of ^{3}H and ^{14}C. Direct addition is generally the method of choice for the determination of ^{3}H and ^{14}C in milk samples.

Table 2. Recommended LSC cocktails and capacities.

Cocktail	Type	Max. capacity at 20 °C*	Comments
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	Safe	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 cow's milk was used throughout.			

Direct addition

Since the composition of milk can vary tremendously, depending upon the species, the data in Table 2 refers to milk obtained from cows.

6. Tissue samples

The sample preparation of tissue samples has been extensively covered in a previous LSC Application Note entitled "LSC Sample Preparation by Solubilization"[3].

Reagents and LSC cocktails

To help with the selection of the appropriate cocktail or reagent, additional information is shown below.

Soluene-350 (Revvity part no. 6003038)

Toluene-based Tissue Solubilizer

0.5 M Quaternary Ammonium Hydroxide in Toluene. Classified as both flammable and corrosive. Recommended cocktails are Hionic-Fluor (classical) and Ultima Gold (safer).

Note: Remember, if Ultima Gold is used in conjunction with Soluene-350, then the mixture is classified as flammable and must be disposed of by incineration.

SOLVABLE (Revvity part no. 6NE9100)

Aqueous-based Tissue Solubilizer

0.4 M Sodium hydroxide in water, plus three specialized surfactants. Classified as corrosive only. Recommended cocktails are Hionic-Fluor & Pico-Fluor Plus (both classical) and Ultima Gold & Opti-Fluor (both safer).

Hionic-Fluor (Revvity part no. 6013319)

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 Plus (Revvity part no. 6013699)

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.

Ultima Gold (Revvity part no. 6013329)

Safer biodegradable cocktail (di-isopropylnaphthalene based), which can accept a wide variety of aqueous sample types. Suitable for use with either Soluene-350 or SOLVABLE. Maximum capacities are 1 mL Soluene-350 or 2 mL SOLVABLE in 10 mL Ultima Gold, with high chemiluminescence resistance.

Opti-Fluor (Revvity part no. 6013199)

Safer biodegradable cocktail (linear alkylbenzene based), designed for use with dilute aqueous sample types at sample volumes up to 2 mL in 10 mL cocktail. Suitable for use with SOLVABLE, but should not be used with Soluene-350.

Table 3. Recommended LSC cocktails and capacities.

Sample type	Sample size	Preparation method	Reagent	Cocktail
Whole blood	Up to 0.5 mL	Solubilization	SOLVABLE	Ultima Gold, Opti-Fluor, Hionic-Fluor, Pico-Fluor Plus
			Solunene-350	Hionic-Fluor, Pico-Fluor Plus, Ultima Gold
Plasma/ serum	Up to 1 mL	Direct Addition		Ultima Gold, Ultima-Flo M
		Solubilization	SOLVABLE	Ultima Gold, Opti-Fluor, Hionic-Fluor, Pico-Fluor Plus
			Soluene-350	Hionic-Fluor, Pico-Fluor Plus, Ultima Gold
Urine	Up to 8 mL	Direct Addition		Ultima Gold, Ultima Gold LLT
Feces	Up to 100 mg	Solubilization	SOLVABLE	Ultima Gold, Opti-Fluor Hionic-Fluor, Pico-Fluor Plus
			Soluene-350	Hionic-Fluor, Pico-Fluor Plus Ultima Gold
			Sodium Hypochlorite	Hionic-Fluor
Milk	Up to 10 mL (see Table 1)	Direct Addition		Opti-Fluor, Pico-Fluor Plus

Summary

A compilation of recommended cocktails for different biological samples is shown in Table 3. This will provide a basic guide to cocktail selection.

Conclusion

There are a variety of LSC cocktails of both safer, high flash point and classical solvent types which are suitable for use in counting biological samples. In addition, a variety of reagents 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 Revvity representative for further applications support.

References

1. Moore, P.A. "Preparation of Whole Blood for Liquid Scintillation Counting." *Clin. Chem.* 27.4 (1981): 609-611.
2. Morrison, B.J. and Franklin, R.A. "A Rapid, Hygenic Method for the Preparation of Fecal Samples for Liquid Scintillation Counting." *Anal. Biochem.* 85 (1978): 79-85.
3. "LSC Sample Preparation by Solubilization." *LSC Application Note*. Revvity. 1996.

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