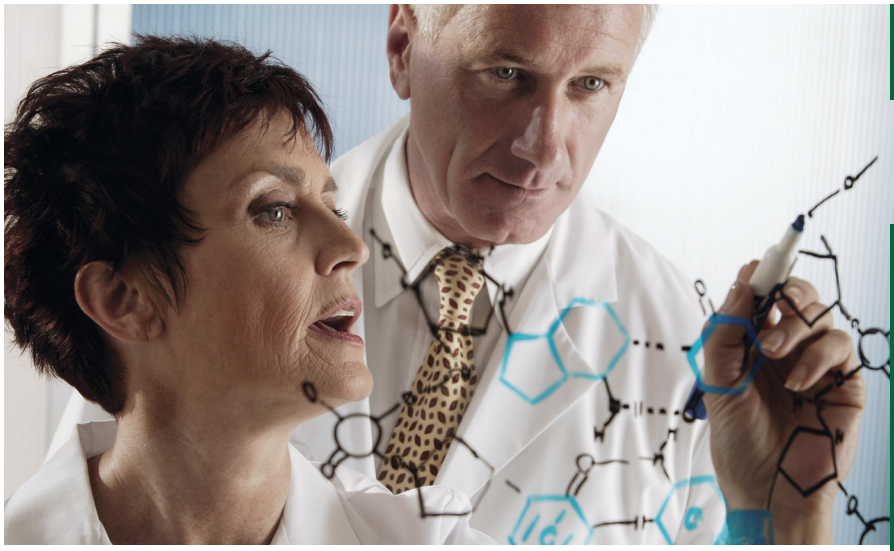

APPLICATION NOTE

Liquid Scintillation

Highlights

- Use of solubilization for various sample types
- Recommended cocktail choices for counting solubilized samples
- Specific sample preparation techniques for various sample types

LSC Sample Preparation by Solubilization

Introduction

In its simplest terms solubilization is the action of certain chemical reagents on the chemical bonds of a macromolecular structure (such as animal or plant tissue) that effects a structural breakdown (or digestion) into smaller, simpler subunits which can then be directly dissolved in a liquid scintillation cocktail[1]. The tissue sample may be whole, homogenized, macerated or in some other state of subdivision prior to solubilization. When the digested samples are added to an appropriate liquid scintillation cocktail they should yield clear, colorless, homogeneous liquids exhibiting a minimum of quench, a minimum of chemiluminescence, and a maximum of counting stability. The chemical reagents used should be capable of rapid and complete digestion with respect to both small and large sample sizes, and should not require any complex care or methodology. Also, the combination of reagents and the method of digestion should allow accurate determination of the isotopic content with a minimum of systematic error. Solubilizers are predominantly used for the traditional animal metabolism studies, and more recently have been increasingly used in cell and tissue culture applications. Another area of growing interest is the fate of radionuclides in the environment and in this field of interest, solubilizers have been found to be an invaluable tool in sample preparation.

Fundamentally there are still only three major classifications of solubilizing reagents (Table 1) and these are:

1. Alkaline Systems (*e.g.* Soluene®-350 and SOLVABLE)
2. Acidic Systems (*e.g.* Perchloric Acid)
3. Other Systems (*e.g.* Sodium Hypochlorite)

The mode of action of "alkaline systems" is solubilization by hydrolysis, and a wide range of samples including animal tissues, blood, urine, bone tissue, muscle, amino acids, nucleic acids and proteins can be digested with these reagents.

With "acidic systems" the sample is oxidized to soluble products by the action of certain strong acids, usually oxidizing acids. Samples such as cartilage, bone, collagen fibers, and dried and hard plant samples can be digested by these reagents. Occasionally mixed acid reagents, and acids with an added oxidizing agent, are preferred due to their increased oxidative power.

Under "other systems" a number of different reagents can be considered, however the most useful reagent is Sodium Hypochlorite whose mode of action is by the process of oxidative bleaching. This is particularly useful when dealing with plant samples, especially those containing chlorophyll, where the Sodium Hypochlorite effectively prevents color quench in subsequent liquid scintillation (LS) counting by bleaching out all of the color present.

It is not possible in this publication to cover the use of every solubilizer and the intention, therefore, is to focus on the most commonly used solubilizers and their usefulness for LSC applications. This paper will identify those sample types which are routinely encountered in solubilization work and will offer helpful hints on sample preparation as well as recommending suitable reagents.

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Table 1. Characteristics of solubilizers.

Reagent	Type	Concentration	Flashpoint	Density (g/mL)	Warning
Soluene®-350	Alkaline#	~ 0.5 M in Toluene	5 °C	0.88	Corrosive, Flammable
Hyamine Hydroxide®	Alkaline#	1.0 M in Methanol	18 °C	0.93	Corrosive, Flammable
SOLVABLE	Alkaline#	0.4 M in Water	—	1.02	Corrosive
Perchloric Acid	Acidic	70%	—	1.7	Corrosive, Oxidative
Nitric Acid	Acidic	68-70%	—	1.42	Corrosive
Sodium Hypochlorite	Other	5-7% (available chlorine)	—	1.16	Corrosive
Hydrogen Peroxide	Other	30% (100 volumes)	—	1.11	Corrosive

(# Quaternary Ammonium Hydroxide type)

Sample Preparation Methods

The following sample preparation techniques, using the reagents detailed in Table 1, were carried out using High Performance Glass Vials. All ^{3}H counting efficiencies presented were determined using a Tri-Carb® 2250 CA with 67% absolute ^{3}H efficiency (sealed argon purged standard) operating at 19 °C.

1. Whole Tissue

The method of solubilizing whole tissue is relatively straightforward, and apart from color formation with certain tissue types, no major problems should be encountered during sample preparation and LS counting. Although this section only mentions Soluene-350 and SOLVABLE, it is also possible in certain cases to use Hyamine Hydroxide®.

(i) Muscle (50 - 200 mg)

The method for processing muscle samples is shown below and typical results are illustrated in Table 2. The choice of LSC cocktail influences the maximum sample size which can be processed.

Procedure

1. Place selected sample size in a 20 mL glass scintillation vial.
2. Add an appropriate volume of solubilizer (1-2 mL depending on sample size).
3. Heat in an oven or water bath at 50 - 60 °C for the specified time with occasional swirling.
4. Cool to room temperature and add 10 mL of a selected cocktail.
5. Temperature and light adapt for at least one hour before counting.

Table 2. Reagents for Solubilization and LS counting of Muscle.

Sample Size	Solubilizer (1 mL used)	Digestion time at 50 – 60 °C	Sample Appearance	LSC Cocktail	^{3}H Counting Efficiency
50 – 200 mg	Soluene-350	$1^1/_2$ – 4 hours	Clear	Hionic-Fluor	41% – 33%
50 – 200 mg	Soluene-350	$1^1/_2$ – 4 hours	Clear	Ultima Gold	49% – 37%
50 – 150 mg	Soluene-350	$1^1/_2$ – 4 hours	Clear	Pico-Fluor Plus	42% – 36%
50 – 200 mg	SOLVABLE	2 – $3^1/_2$ hours	Clear	Hionic-Fluor	42% – 33%
50 – 200 mg	SOLVABLE	2 – $3^1/_2$ hours	Clear	Ultima Gold	48% – 41%
50 – 150 mg	SOLVABLE	2 – $3^1/_2$ hours	Clear	Pico-Fluor Plus	39% – 34%

Table 3. Reagents for Solubilization and LS counting of Liver.

Sample Size	Solubilizer (1 mL used)	Digestion time at 50 – 60 °C	Sample Appearance#	LSC Cocktail	^{3}H Counting Efficiency
100 mg	Soluene-350	4 hours	Clear yellow	Hionic-Fluor	15%
50 mg	SOLVABLE	1 hour	Clear, slight yellow tinge	Ultima Gold	47%
100 mg	SOLVABLE	$^1/_2$ hours	Clear, light yellow	Hionic-Fluor	23%

(#Appearance after decolorization with hydrogen peroxide)

(ii) Liver (50 - 100 mg)

The method for processing liver samples is shown below and typical results obtained are illustrated in Table 3. As before, the choice of LSC cocktail influences the maximum sample size which can be processed.

Procedure

1. Place selected sample size in a 20 mL glass scintillation vial.
2. Add 1-2 mL of solubilizer.
3. Heat in an oven or water bath at 50 - 60 °C for the specified time with occasional swirling.
4. Cool to room temperature.
5. Add 0.2 mL of 30% hydrogen peroxide in two aliquots of 0.1 mL, with swirling between additions. Allow any reaction to subside between additions of the hydrogen peroxide.
6. Heat again at 50 - 60 °C for 30 minutes to complete decolorization.
7. Add 10 mL of a selected cocktail and temperature and light adapt for at least one hour before counting.

Notes

Solubilization of liver always results in highly colored samples due to the presence of bilirubin. The above work was therefore restricted to a viable sample size (which should ideally not exceed 75 mg). In our experience, SOLVABLE has proved to be better than Soluene-350 for this particular sample type, mainly due to more rapid solubilization.

(iii) Kidney / Heart / Sinew / Brains / Stomach Tissue

The method for processing the above five sample types is shown below and typical results are illustrated in Table 4. As previously stated, the choice of LSC cocktail influences the maximum sample size which can be processed.

Procedure

1. Place selected sample size in a 20 mL glass scintillation vial.
2. Add 1-2 mL of solubilizer.
3. Heat in an oven or water bath at 50 - 60 °C for the specified time with occasional swirling.
4. Cool to room temperature.
5. Add 0.2 mL of 30% hydrogen peroxide in two aliquots of 0.1 mL with swirling between additions. Allow any reaction to subside between additions of hydrogen peroxide.
6. Heat again at 50 - 60 °C for 30 minutes to complete decolorization.
7. Add 10 mL of selected cocktail, temperature and light adapt for one hour before counting.

Note

It is not possible to digest stomach tissue with SOLVABLE.

Table 4. Reagents for solubilization and LS counting of various tissues.

Sample Size	Solubilizer (1 mL used)	Digestion time at 50 – 60 °C	Sample Appearance#	LSC Cocktail	^{3}H Counting Efficiency
Kidney 50 – 100 mg	Soluene-350	$1^1/_2$ – 5 hours	Clear, slight yellow	Hionic-Fluor	41% – 34%
50 – 100 mg	SOLVABLE	1 – 2 hours	Clear, slight yellow	Hionic-Fluor	40% – 38%
Heart 50 – 100 mg	Soluene-350	2 – 3 hours	Clear, slight yellow	Hionic-Fluor	40% – 38%
50 – 150 mg	SOLVABLE	1 – 3 hours	Clear, slight yellow	Hionic-Fluor	40% – 38%
Sinew 50 – 150 mg	Soluene-350	1 – 4 hours	Clear, slight yellow	Hionic-Fluor	44% – 38%
50 – 150 mg	SOLVABLE	1 – 2 hours	Clear, slight yellow	Hionic-Fluor	42% – 39%
Brains 50 –150 mg	Soluene-350	$1^1/_2$ – 2 hours	Clear, slight yellow	Hionic-Fluor	43% – 41%
50 –150 mg	SOLVABLE	1 – 2 hours	Clear, slight yellow	Hionic-Fluor	42% – 40%
Stomach 50 – 100 mg	Soluene-350	$2^1/_2$ – 3 hours	Clear, very slight yellow tinge	Hionic-Fluor	41% –– 39%

(#Appearance after decolorization with hydrogen peroxide)

(iv) Feces

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 rabbit. 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 and isotope recoveries of greater than 98% for ^{3}H were achieved. This recovery level was confirmed by combustion in a Sample Oxidizer (PerkinElmer Sample Oxidizer, Model 307). The solubilization method used for processing this feces sample is shown below.

Procedure

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 one hour before counting.

Note

After digestion, a small amount of white residual matter may remain, however this should not affect the recovery.

2. Blood

The successful preparation of blood samples[3] for LS counting 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. Consequently, methods are given for both Soluene-350 and SOLVABLE, and the final choice of method rests with the individual researcher. Some typical results, obtained in our own laboratories, are shown in Table 5.

(i) Soluene-350 Method

Procedure

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 (1:1 or 1:2 ratio). Ethanol may be substituted for the isopropyl alcohol 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.
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 15 mL of Hionic-Fluor.
9. Temperature and light adapt for one hour before counting.

(ii) SOLVABLE Method

Procedure

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 one hour. Sample at this stage will be brown/green in appearance
4. Add 0.1 mL of 0.1M 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.
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 one hour. The color will change from brown/green to pale yellow.
8. Cool to room temperature and add 15 mL of Pico-Fluor Plus.
9. Temperature and light adapt for one hour before counting.

3. Plant Material

There are two main problems associated with digestion of plant material: the presence of pigments (especially chlorophyll), and the difficulty of digesting cellulose. Some of the colored pigments can be bleached with hydrogen peroxide, but not all, and therefore many samples will remain highly colored. The primary problem with cellulose is that it is not soluble in the alkaline solubilizers, and, in general, some form of skeletal cellulose material remains after attempted solubilization. To overcome these two problems a number of different reagent systems have been devised with the overall result that plant material can be processed. Since there is such a wide variety of plant sample types, this section will be confined to describing the solubilization methods together with their associated advantages and drawbacks.

Table 5. Reagents for solubilization and LS counting of blood.

Sample Size	Solubilizer	Sample Appearance#	LSC Cocktail	^{3}H Counting Efficiency
0.20 - 0.4 mL	Soluene-350	Clear, pale yellow	Hionic-Fluor	29% - 19%
0.25 - 0.5 mL	SOLVABLE	Clear, pale yellow	Hionic-Fluor	37% - 27%
0.50 mL	SOLVABLE	Clear, pale yellow	Pico-Fluor Plus	29%
0.20 mL	SOLVABLE	Clear, pale yellow	Ultima Gold	44%

(#Sample Appearance after decolorization with hydrogen peroxide)

(i) Soluene-350

Soluene-350 can be used to solubilize various plant materials, but in general the sample size must be kept small (< 50 mg). With such small samples it is often possible to achieve limited decolorization with hydrogen peroxide; however, color quenching remains a problem and the cellulose is not dissolved. This classifies the use of Soluene-350 for the digestion of plant material in the "of limited use" category.

(ii) SOLVABLE

SOLVABLE is also not ideally suited to solubilizing plant materials and suffers from the same drawbacks associated with Soluene-350.

(iii) Perchloric Acid/Nitric Acid Solution and Perchloric Acid/ Hydrogen Peroxide Solution

These two reagent systems digest samples by the method of "wet oxidation" and are particularly useful for solubilizing samples such as hard and dried plant material. They have also proved useful for the digestion of cartilage, bone, collagen fibers and even some highly colored samples such as blood and liver. The general methods for each reagent system are as follows :

Perchloric Acid / Nitric Acid[4]

Procedure

1. Prepare the solubilizing reagent by adding one volume of 70% perchloric acid to one volume of 70% nitric acid.
2. Where possible the sample should be oven dried and then finely cut.
3. Place prepared sample (up to 200 mg) in a glass scintillation vial fitted with a poly-cone lined urea screw cap.
4. Add approximately 0.6 mL of the prepared $HClO_4/HNO_3$ reagent (1:1).
5. Digest the sample in the closed vial at 50 - 70 °C for one hour or until an almost colorless solution is obtained.
6. Cool the vial to room temperature and add 15 mL of Hionic-Fluor.
7. Temperature and light adapt for one hour before counting.

Perchloric Acid / Hydrogen Peroxide[5,6,7,8]

Procedure

1. Where possible the sample should be oven dried and then finely cut.
2. Place prepared sample (up to 200 mg) in a glass scintillation vial fitted with a poly-cone lined urea screw cap.
3. Add 0.2 mL of 70% perchloric acid and swirl gently to completely wet the sample.
4. Add 0.4 mL of 30% hydrogen peroxide and again swirl gently. (This sequence of addition prevents frothing.)
5. Digest the sample in a closed vial at 50 - 70 °C for one hour or until an almost colorless solution is obtained.
6. Cool the vial to room temperature and add 15 mL Hionic-Fluor.
7. Temperature and light adapt for one hour before counting.

Notes (for both methods)

With both reagent systems, if the isotope label is ^{14}C, there is a potential for loss of the label as radiocarbon dioxide ($^{14}CO_2$) during solubilization. Tritium (^{3}H) losses are virtually prevented due to the formation of $^{3}H_2O$ which condenses inside the vial. Ensure that a poly-cone insert is used in the cap in place of a foil lined insert as these aggressive reagents will oxidize the foil (aluminium) insert and may contaminate the digest. Do not heat these mixture above 90 °C; perchloric acid may decompose violently above this temperature. Due to the aggressive nature of these mixtures the use of gloves is recommended during all handling steps. The perchloric acid/ nitric acid method has also been successfully used for the determination of ^{45}Ca and ^{35}S in cartilage and bone (unpublished work).

(iii) Sodium Hypochlorite[8,9,10]

Although sodium hypochlorite does not completely dissolve cellulose, it is capable of decolorizing not only chlorophyll but also almost all other pigments found in plant materials. Therefore, providing that the radioisotope is not assimilated within the cellulose structure, this reagent should be considered for preparing plant materials for LS counting. The advantages of using this reagent for plant solubilization are that it is simple, rapid and does not result in loss of ^{14}C as radiocarbon dioxide gas. In practice, sodium hypochlorite penetrates the plant material and rapidly decolorizes the pigments, leaving behind a white skeleton of cellulose material. The general method is described below.

Procedure

1. Place the sample (up to 200 mg) in a glass scintillation vial.
2. Add 1.0 mL of sodium hypochlorite solution.
3. Swirl gently until all of the sample has been completely wetted.
4. Cap tightly and place in an oven or water bath at 50 - 60 °C for approximately one to two hours.
5. Completeness of digestion is usually indicated by removal of pigmentation and/or the appearance of white skeleton of cellulose.
6. Cool the vial to room temperature and carefully vent each vial under a fume hood (decolorization is by action of released chlorine and some residual chlorine remains).
7. Blow out any remaining chlorine with a gentle stream of air or nitrogen.
8. Add 15 mL Hionic-Fluor.
9. Temperature and light adapt for one hour before counting.

Note

Sodium hypochlorite is more commonly known as bleach and should have greater than 5% available chlorine if it is to be an effective solubilizer.

Summary

The information presented in the previous sections (1-3) of this publication is condensed into a quick reference guide (Table 6). This may prove particularly useful in selecting the most appropriate solubilizer for a specific sample type.

Conclusion

There are a variety of PerkinElmer LSC cocktails, of both high flash-point and of classical solvent types, which are suitable for use with the various solubilizing reagents. If problems with solubilizing and LS counting persist, or help is needed with an alternative solubilizer not mentioned in this publication, please call your local PerkinElmer representative for further applications support.

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Table 6. Solubilization selection guide.

Sample Type	Solubilizer	Maximum sample size	Suitable LSC Cocktails
Muscle	Soluene-350	150 mg 200 mg	Pico-Fluor Plus Ultima Gold or Hionic-Fluor
	SOLVABLE	150 mg 200 mg	Pico-Fluor Plus Ultima Gold or Hionic-Fluor
Liver	Soluene-350	100 mg	Hionic-Fluor
	SOLVABLE	50 mg 100 mg	Ultima Gold Hionic-Fluor
Kidney	Soluene-350	100 mg	Hionic-Fluor
	SOLVABLE	100 mg	Hionic-Fluor
Heart	Soluene-350	100 mg	Hionic-Fluor
	SOLVABLE	150 mg	Hionic-Fluor
Sinew	Soluene-350	150 mg	Hionic-Fluor
	SOLVABLE	150 mg	Hionic-Fluor
Brains	Soluene-350	150 mg	Hionic-Fluor
	SOLVABLE	150 mg	Hionic-Fluor
Stomach	Soluene-350	100 mg	Hionic-Fluor
Feces	Hypochlorite	150 mg	Hionic-Fluor
	Soluene-350	20 mg	Hionic-Fluor
	SOLVABLE	20 mg	Hionic-Fluor
Blood	Soluene-350	0.4 mL	Hionic-Fluor
	SOLVABLE	0.5 mL 0.2 mL	Hionic-Fluor or Pico-Fluor Plus Ultima Gold
Plant Material	Soluene-350	< 50 mg	Hionic-Fluor
	SOLVABLE	< 50 mg	Hionic-Fluor
	$HClO_4/H_2O_2$	200 mg	Hionic-Fluor
	$HClO_4/HNO_3$	200 mg	Hionic-Fluor
	Sodium Hypochlorite	200 mg	Hionic-Fluor

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