MERIDIAN BIOTECHNOLOGIES Ltd
Vials & Cocktails for Liquid Scintillation

LSC Sample Preparation by Solubilisation

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Contents

Introduction

In its simplest terms solubilisation 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. The tissue sample may be whole, homogenized, macerated or in some other state of subdivision prior to solubilisation. When the digested samples are added to an appropriate liquid scintillation cocktail, they should yield clear, colourless, 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. Solubilisers are predominantly used for the traditional animal metabolism studies, and more recently have been increasingly used in cell and tissue culture applications.

The most common type of solubiliser is an alkaline reagent and there are two versions currently available. By far the most popular is a quaternary ammonium hydroxide and there are two distinct versions:-

GoldiSol (Meridian Biotechnologies Ltd.)
Quaternary ammonium hydroxide in iso-propyl alcohol / water.
Classified as flammable and corrosive.

Soluene® 350 (Perkin Elmer Inc)
Quaternary ammonium hydroxide in toluene / methanol.
Classified as toxic, flammable and corrosive.

In addition, there are aqueous based solubilisers that can be substituted for the quaternary ammonium hydroxide system in almost all cases. Only two are available:-

AquiGest (Meridian Biotechnologies Ltd)
Classified as corrosive.

Solvable™ (Perkin Elmer Inc)
Classified as corrosive.

The properties of the above are summarised in the following table:

Reagent	Type	Concentration	Flash-point	Density (g/mL)	Warning
GoldiSol	Alkaline	~ 0.5 M in Iso-propyl alcohol / water	12°C	0.83	Corrosive, Flammable
Soluene®350	Alkaline	~ 0.5 M in Toluene / Methanol	5°C	0.88	Toxic, Corrosive, Flammable
AquiGest	Alkaline	0.5 M in Water	--------	1.02	Corrosive
Solvable™	Alkaline	0.4 M in Water	--------	1.02	Corrosive

Sample Preparation Methods

Before giving detailed sample preparation methods for different sample types it is best that a general synopsis of the method is given with explanations about each step.

1. Place the selected sample size in a 20 mL glass scintillation vial.
It is important to use a sample size within the given recommendations. For larger sample sizes the volume of solubiliser must be increased on a pro rata basis. A glass scintillation vial is recommended for GoldiSol. Avoid using a plastic vial as the solvent can permeate through the wall and produce a potentially explosive atmosphere. However, a plastic scintillation vial may be used with ProSafe TS+ and AquiGest

2. Add an appropriate volume of solubiliser (1-2 mL depending on sample size).
It is important to use a sample size within the given recommendations. For larger sample sizes the volume of solubiliser must be increased on a pro rata basis.

3. Heat in an oven or water bath at 50° to 60°C for the specified time with occasional swirling.
The rate of solubilisation is dependent upon temperature and since the rate of a chemical reaction is inversely proportional to temperature then the greater the temperature the more rapid the solubilisation. However, do not exceed 65°C. Occasional swirling is advised to keep the sample in contact with the solubiliser –some samples stick to the glass wall and need to be free in solution.

4. Cool to room temperature

5. Add 0.2 to 0.5 mL of 30% hydrogen peroxide in aliquots of 0.1 mL. Swirl between additions and allow any reaction (frothing) to subside between additions of hydrogen peroxide.
Hydrogen peroxide (30% or 100 vols) is added to decolorize the solubilisate. When using GoldiSol there is a minimum of frothing. If Soluene®350 is used there will be considerable frothing – take care.

6. Heat again at 50° to 60°C for 30 minutes to complete decolourisation.
This completes decolourisation and destroys any excess hydrogen peroxide.

7. Add the appropriate volume of selected cocktail and temperature and light adapt for at least 30 minutes before counting.
Temperature and light adaptation, in the LSC counter, allows any residual chemiluminescence and photoluminescence to decay and assures consistent counting.

The following sample preparation methods are well tried-and-tested and have been used successfully for many years. Since not all biological samples are the same, they have been subdivided into groups that have the same methodologies.

Whole blood
Muscle, Skin, Heart, Stomach, Brains, Stomach tissue
Liver & Kidney
Fatty tissue
Feces

Whole blood

Because of its complex nature and extreme colour, blood should not be added directly to a cocktail. Such an attempt will result in a highly colour quenched and non-homogeneous mixture that will not lend itself to accurate or reproducible counting. To prepare blood samples for counting treatment by solubilisation (digestion) is required. Solubilisation is the action of certain chemical reagents on organic materials (such as animal tissue) that effects a structural breakdown (or digestion) into a liquid form that can then be directly dissolved in a liquid scintillation cocktail. The source of blood and the correct choice of solubiliser will significantly 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, there can still be colour quench problems.

The successful preparation of blood samples for LSC counting by solubilisation can often be technically difficult, and successful digestion can be largely dependent on the practical experience of the researcher. To process a sample simply add the solubiliser and heat at 55° to 60°C until the sample is dissolved. After solubilisation the sample may be coloured and this colour can usually be removed or reduced by treatment with hydrogen peroxide. The final step is to add the recommended LSC cocktail and the sample is ready for counting. Using this method many samples can be processed simultaneously and then counted sequentially. An aqueous based solubiliser such as AquiGest is the recommendation for whole blood. The respective stepwise processes for solubilisation with these different solubilisers are as follows:-

Aqueous based solubiliser

1. Add a maximum of 0.4 mL blood to a glass scintillation vial.
2. Add 1.0 mL solubiliser.
3. Incubate the sample at 55° to 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. This reagent also complexes iron that has been released during digestion and produces a mixture that is much less coloured.
5. Add 0.3 mL to 0.5 mL of 30% hydrogen peroxide in 100 µL aliquots. Slight foaming will occur after each addition; therefore, gentle agitation is necessary. Keep swirling the mixture until all foaming subsides and then repeat the process until all 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 55° to 60°C for one hour. This is important as heating destroys residual hydrogen peroxide which if present can induce unwanted chemiluminescence. The colour will change from brown/green to pale yellow.
8. Cool to room temperature and add 15 mL of recommended LSC cocktail.
9. Temperature and light adapt for one hour before counting.

Quaternary ammonium hydroxide 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 solubiliser and isopropyl alcohol (1**:**1 or 1**:**2 ratio). Ethanol may be substituted for the isopropyl alcohol if desired.
3. Incubate at 55° to 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 in 100 µL aliquots. Foaming may occur after each addition; therefore, gentle agitation is necessary. Keep swirling the mixture until all foaming subsides and then repeat the process until all 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 55° to 60°C for 30 minutes. The samples at this stage should now have changed to slightly yellow. This is important as

heating destroys residual hydrogen peroxide which if present can induce unwanted chemiluminescence.
8. Cool to room temperature and add 15 mL of recommended LSC cocktail.
9. Temperature and light adapt for one hour before counting.

Both the above methods apply to not only blood but also to any other biological tissue sample and the only modifications required concern the sample sizes. Since the products of such solubilisation are in an alkaline mixture there is a high degree of probability that chemiluminescence may be present. Whenever a new method is being evaluated it is strongly recommended that checks are carried out to confirm that the solubilisers and LSC cocktails do not produce chemiluminescence. This check should also be carried out on new deliveries of LSC cocktail to confirm quality. To determine if there is a potential problem follow the steps below:-

1. Add the appropriate volume of selected cocktail to a glass scintillation vial.
2. Add the appropriate volume of solubiliser (1 or 2 mL).
3. Shake thoroughly to mix.
4. Place in the counter and arrange for repeat 2 minute counts.
5. If normal background levels are observed (<25 CPM in a standard Tritium window) there is no chemiluminescence.
6. If higher than expected CPMs are observed continue repeat counting till CPM returns to normal background level.
7. Calculate total time taken for CPM stabilization and this becomes the fixed period the sample needs to stand before starting to count after the solubilisation process.

Chemiluminescence is produced by an interaction between free peroxides and alkaline material. The free peroxides originate from the cocktail and more specifically from the ethoxylate detergents present in the cocktail. Over time the ethoxylate polymer chain breaks down and the result is a free peroxide entity. In general, freshly manufactured LSC cocktail is peroxide-free but with prolonged storage the level of peroxide will increase and therefore attention should be given to the age of the cocktail being used in solubilisation studies.
Although blood is a complex mixture and solubilisation is an involved process it is possible to successfully process and count blood samples both accurately and reproducibly. It is critical that quench curves be used to record activity in DPM due to the variable quench present in solubilised samples.

Muscle, Skin, Heart, Stomach, Brains, Stomach tissue

The method of solubilising all the above is relatively straightforward, and apart from possible colour formation, no major problems should be encountered during sample preparation and LSC counting. The method can be considered a general method and is as follows:-

1. Place selected sample (up to 200 mg) in a 20 mL glass scintillation vial.
2. Add an appropriate volume of solubiliser (up to 100 mg use 1 mL: 100 to 200 mg use 2 mL).
3. Heat in an oven or water bath at 55° to 60°C with occasional swirling until the sample is completely dissolved.
4. Cool to room temperature.
5. If colour is present add 100 to 200 µL of 30% (100 vol) hydrogen peroxide and allow to stand for 10 to 15 minutes or until any reaction has subsided. If no colour is present proceed to step 7.
6. Heat again at 55° to 60°C for 30 minutes to complete decolourization.
7. Add 10 mL of a recommended cocktail.
8. Temperature and light adapt for at least one hour before counting.

Although all these samples can be solubilised using either the organic or aqueous based solubilisers it is important to note that there are a few exceptions.

There are two samples that cannot be solubilised with the aqueous based solubiliser and these are arteries and veins, and stomach tissue. Both sample types are only very slowly or partially solubilised by an aqueous based solubiliser and for complete solubilisation the quaternary ammonium hydroxide type must be used.

Liver & Kidney

Both liver and kidney samples are brownish in colour and in addition contain blood or blood decomposition products. Consequently, sample preparation by solubilisation will be similar to that used for whole blood and as with blood it is possible to use both solubilisers mentioned previously. AquiGest is the recommended solubiliser for liver and kidney
The method for liver and kidney is as follows:-

Aqueous based solubiliser

1. Add a maximum of 100mg liver or kidney to a glass scintillation vial.
2. Add 1.0 mL solubiliser.
3. Incubate the sample at 55° to 60°C for one hour.
4. Add 0.1 mL of 0.1M EDTA-di-sodium salt solution which helps reduce foaming when the subsequent hydrogen peroxide is added. This reagent also complexes iron that has been released during digestion and produces a mixture that is less coloured.
5. Add 0.3 mL to 0.5 mL of 30% hydrogen peroxide in 100 µL aliquots. Slight foaming will occur after each addition; therefore, gentle agitation is necessary. Keep swirling the mixture until all foaming subsides and then repeat the process until all 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 55° to 60°C for one hour. This is important as heating destroys residual hydrogen peroxide which if present can induce unwanted chemiluminescence. The colour will change to pale yellow.
8. Cool to room temperature and add 15 mL of recommended LSC cocktail.
9. Temperature and light adapt for one hour before counting.

Quaternary ammonium hydroxide method

1. Add a maximum 100mg liver or kidney to a glass scintillation vial.
2. Add 1.0 mL of selected solubiliser.
3. Incubate at 55° to 60°C for 2 hours.
4. Cool to room temperature.
5. Add 0.2 mL to 0.5 mL of 30% hydrogen peroxide in 100 µL aliquots. Foaming will occur after each addition; therefore, gentle agitation is necessary. Keep swirling the mixture until all foaming subsides and then repeat the process until all 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 55° to 60°C for 30 minutes. The samples at this stage will now be slightly yellow. This is important as heating destroys residual hydrogen peroxide which if present can induce unwanted chemiluminescence.
8. Cool to room temperature and add 15 mL of recommended LSC cocktail.
9. Temperature and light adapt for one hour before counting.

Solubilisation of liver and kidney always results in highly coloured samples due to the presence of bilirubin and in addition such samples from small animals will produce even more colour. It is strongly recommended that sample size should not exceed 75mg for small animal samples. The aqueous based solubiliser has proved to be better than the quaternary ammonium hydroxide solubiliser for these particular sample types, mainly due to significantly less colour development at the end of the solubilisation process. If excessive colour still develops then one way of reducing the interference is to increase the volume of LSC cocktail from 15mL to 20mL. In effect this is diluting the colour but it is a solution to the problem. Due to the presence of colour quenching it is critical that quench curves be used to record activity in DPM. Do not work with CPM as these will vary from sample to sample.

Fatty tissue

Due to the chemical nature of fats they are difficult to solubilize and only the **quaternary ammonium hydroxide type solubilisers are capable of solubilising fatty tissue.** Even then the rate of solubilisation is slow and may require up to 24 hours to completely solubilize animal fatty tissue. Fat samples can be completely solubilised but it is a matter of time, temperature and sample condition. Recommendations include:-

- cutting the fat samples into small pieces which increases the surface area and hence the rate of reaction
- carrying out the solubilisation at 60° to 65°C
- adding a small amount of IPA (isopropyl alcohol)

Other than the rate of solubilisation the only other problem that will occur is colour formation. Solubilisation of fatty tissue almost invariably results in yellow colouration of the final mixture. The method is as follows:-

1. Add a maximum 100 mg finely divided fat to a glass scintillation vial.
2. Add 1.0 mL of selected solubiliser and optionally 1.0mL IPA.
3. Incubate at 60° to 65°C until the entire fat sample is solubilised (may take 10 to 15 hours).
4. Cool to room temperature.
5. Add 0.2 mL to 0.5 mL of 30% hydrogen peroxide in 100 µL aliquots. Foaming will occur after each addition; therefore, gentle agitation is necessary. Keep swirling the mixture until all foaming subsides and then repeat the process until all 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 55° to 60°C for 30 minutes. The samples at this stage will now be slightly yellow. This is important as heating destroys residual hydrogen peroxide which if present can induce unwanted chemiluminescence.
8. Cool to room temperature and add 15 mL of recommended LSC cocktail.
9. Temperature and light adapt for one hour before counting.

As before, due to the presence of colour quenching it is critical that quench curves be used to record activity in DPM. Do not work with CPM as these will vary from sample to sample.

Feces

The digestion of feces strongly depends on the type of animal. It is possible to use both the quaternary ammonium hydroxide type and the aqueous based solubilisers but there can be problems with residual colour and incomplete digestion due to cellulose type material present in feces from species such as rabbit. The method is the same as shown previously for Muscle, Skin, Heart, Stomach, Brains and Stomach tissue.

1. Place selected sample (up to 100 mg) in a 20 mL glass scintillation vial.
2. Add 1.0 mL of selected solubiliser.
3. Heat in an oven or water bath at 50° to 60 °C with occasional swirling until the sample is completely dissolved.
4. Cool to room temperature.
5. If colour is present add 100 to 200 µL of 30% (100 vol) hydrogen peroxide and allow to stand for 10 to 15 minutes or until any reaction has subsided. If no colour is present proceed to step 7.
6. Heat again at 50° to 60°C for 30 minutes to complete decolourisation.
7. Add 10 mL of a recommended cocktail.
8. Temperature and light adapt for at least one hour before counting.

After digestion, a small amount of white residual matter (most probably undigested cellulose) may remain, however this should not affect the recovery.

Summary

A summary of all these methods, including recommended LSC cocktails is given in the table below:-

Sample type	**Solubiliser**	**Max. Sample size**	**Recommended LSC cocktail**
Muscle	GoldiSol	200 mg	ProSafe TS
	AquiGest	200 mg	ProSafe TS
Liver	GoldiSol	100 mg	ProSafe TS
	AquiGest	100 mg	ProSafe TS
Kidney	GoldiSol	100 mg	ProSafe TS
	AquiGest	100 mg	ProSafe TS
Heart	GoldiSol	100 mg	ProSafe TS
	AquiGest	150 mg	ProSafe TS
Sinew	GoldiSol	150 mg	ProSafe TS
	AquiGest	150 mg	ProSafe TS
Brains	GoldiSol	150 mg	ProSafe TS
	AquiGest	150 mg	ProSafe TS
Stomach	Soluene®350	100 mg	ProSafe TS
Faeces	GoldiSol	100 mg	ProSafe TS
	AquiGest	100 mg	ProSafe TS
Blood	GoldiSol	0.4 mL	ProSafe TS
	AquiGest	0.4 mL	ProSafe TS

For further information please ring
0208 397 8316 or email info@meridian-biotech.com

ProSafe TS+ was developed to work with solubilised samples.
ProSafe TS+ will accept samples solubilised with either GoldiSol or AquiGest.
ProSafe TS+ is also able to accept commonly encountered media used in general LSC counting.

ProSafe TS+:

- Suitable for use samples solubilised with all available solubilisers
- Suitable for use with other buffers/media
- NPE-free cocktail making it more environmentally friendly.
- High flash point (147°C) increases safety
- Can be used safely on the bench

ProSafe TS+	Part number: PSTS+	2 x 5 Litre
Goldisol	Part number GTS05	500 mL
AquiGest	Part number AGS05	500 mL