Counting aqueous samples by LSC.

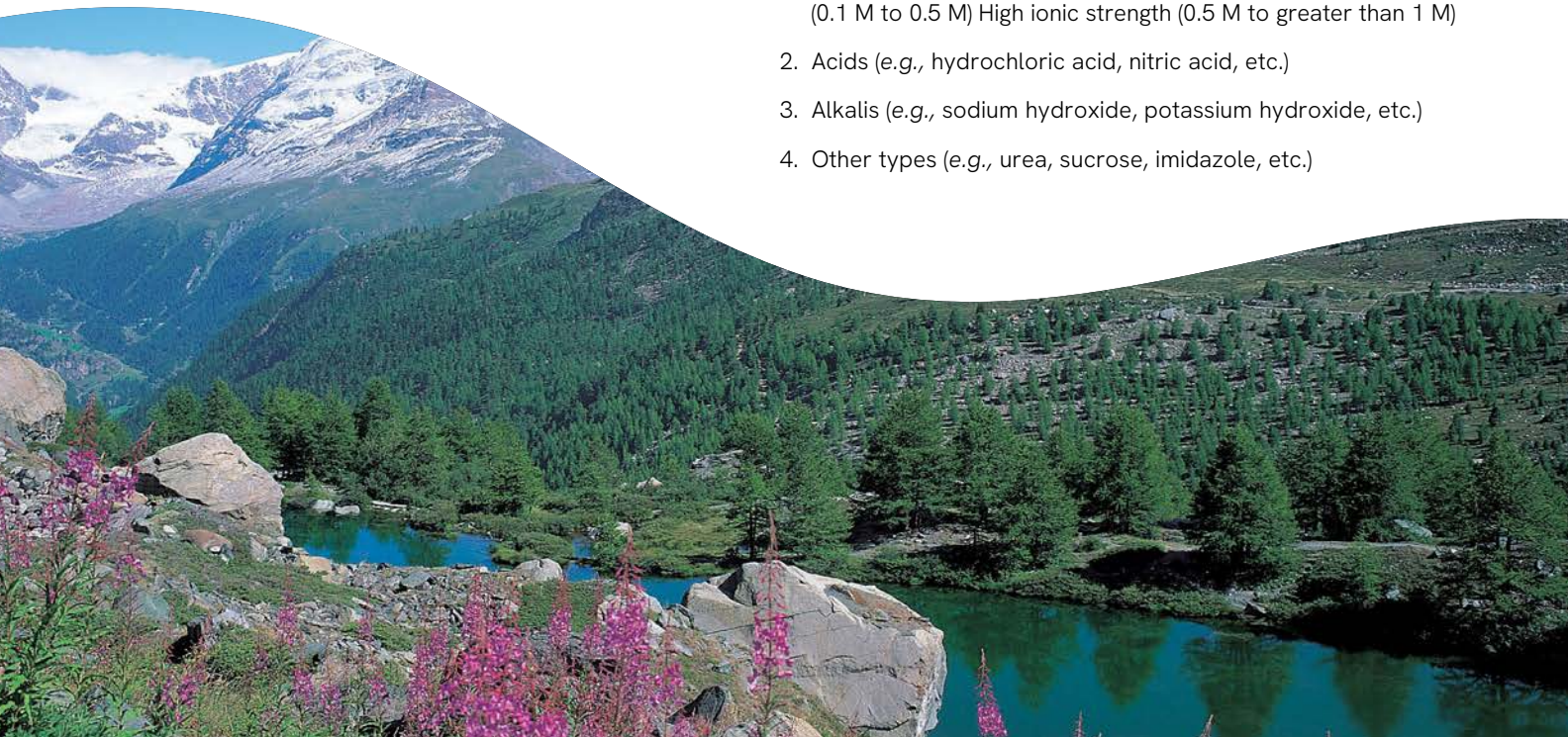
Highlights

- Cocktail selection for various ionic strength buffers
- Cocktail selection for acids and alkalis
- Samples capacities for classical and safer cocktails

Introduction

The primary objective of all sample preparation procedures for liquid scintillation counting (LSC) is to obtain a homogeneous solution for efficient energy transfer from the sample to the LS cocktail. Aqueous solutions are some of the simplest and most commonly found in liquid scintillation analysis. In general, they provide the environment necessary for many assays and separations, and include the most encountered solvent media for the numerous radioisotopes used in LSC. The main methods for producing aqueous samples are by dissolution, extraction, and distillation. Dissolution simply involves dissolving the sample in water. Extraction can be extraction of the sample from a solid matrix by water (solid/liquid extraction), or extraction of the sample from a liquid matrix by water (liquid/liquid extraction). Distillation involves separation of the aqueous component by evaporation. A variety of LS cocktails have evolved over the years to accommodate the diverse types, volumes, and concentrations of aqueous samples presented for analysis by LSC.[1,2,3] Different types of anions encountered in LSC include chlorides, nitrates, phosphates, acetates, and formates, with sample volumes ranging from less than 100 µL to greater than 10 mL, and concentrations varying from less than 10 mM to greater than 2 M. For the purpose of cocktail selection, these aqueous samples can be roughly divided into the following categories:

1. Buffers (*e.g.,* sodium chloride, PBS, potassium phosphate, etc.) Low ionic strength (less than 0.1 M) Medium ionic strength (0.1 M to 0.5 M) High ionic strength (0.5 M to greater than 1 M)
2. Acids (*e.g.,* hydrochloric acid, nitric acid, etc.)
3. Alkalis (*e.g.,* sodium hydroxide, potassium hydroxide, etc.)
4. Other types (*e.g.,* urea, sucrose, imidazole, etc.)



By using this list of categories, it is now possible to assign cocktails for each category and therefore present a simpler and more comprehensive method of cocktail selection than was previously possible. For each category, cocktails will be recommended based on sample acceptance, performance and safety.

Buffers – Low ionic strength

Buffers encountered in this group include 0.01 M PBS (phosphate buffered saline), 50 mM Tris-HCl [Tris(hydroxymethyl)aminoethane hydrochloride], 0.1 M NaCl (sodium chloride), 0.01 M Na_2SO_4 (sodium sulphate), etc. Since these aqueous buffers are relatively dilute, there are comparatively few problems. Both di- and tri-valent anions such as SO_4^{2-} and PO_4^{3-} are potentially problematic, due in part to their charge and in part to their relative size [*e.g.,* chlorides (Cl^-) are much smaller than sulphates (SO_4^{2-})]. These characteristics can impede the formation of a stable microemulsion and can cause phase instability, especially with high concentrations and large volumes. Surprisingly, similar problems can occur with small volumes, particularly within the range of 0.1 mL to 0.5 mL sample in 10 mL cocktail (1 to 5% sample load). The only other area of concern is color quench problems when using certain metallic salts which are intrinsically colored [*e.g.,* $FeCl_3$ (ferric chloride)]. Any phase instability problem can usually be resolved by decreasing the sample volume or by increasing the cocktail volume. If the problem persists, then it may be necessary to change to a cocktail which can accept higher strength ionic samples. Color quench problems can be reduced by either diluting the sample with water (if practicable), or by using a cocktail which is more resistant to color quenching *e.g.,* any of the Ultima Gold cocktails.

Among the safer cocktails, the Ultima Gold family [based on DIN (di-isopropylnaphthalene)] will give higher ^3H efficiency than Opti-Fluor or Emulsifier-Safe [based on LAB (linear alkyl benzene)]. Of the classical solvent-based cocktails, Insta-Gel Plus (10 mL) can accommodate greater than 2.5 mL of certain sample types and forms a stable gel (usually at greater than 3 mL sample volume), thus making large sample volumes possible. Pico-Fluor Plus (10 mL) can accommodate greater than 2.5 mL of certain samples and remains in a single liquid phase. Cocktails suitable for these samples are shown in Table 1.

Buffers – Medium ionic strength

Aqueous buffer concentrations encountered in this group range from 0.1 M up to 1.0 M and typical buffers are 0.1 M to 0.5 M PBS, 0.15 M to 1.0 M NaCl, 0.25 M ammonium acetate, etc. The cocktails suitable for these sample types (shown in Table 2) are designed to overcome any phase instability problems and therefore cocktail selection is limited by volume and concentration factors.

The Ultima Gold family will, in general, give higher quench resistance than the classical cocktails i.e., higher efficiency at the same sample load. Ultima Gold works well with low sample volumes of aqueous buffers up to 0.5 M. Although Insta-Gel Plus will accept small volumes of certain sample types, Pico-Fluor Plus is the recommended classical cocktail for these samples.

Table 1: Cocktail selection for low ionic strength samples (based on the use of 10 mL of cocktail).

	Safer cocktail	Classical cocktail
Low sample volume (0 to 2.5 mL)	Ultima Gold	Insta-Gel Plus
	Ultima Gold MV	Bio-Fluor Plus
	Ultima Gold AB	Pico-Fluor Plus
	Ultima Gold LLT	
	Opti-Fluor	
	Emulsifier-Safe	
High sample volume (>2.5 mL)	Ultima Gold	Insta-Gel Plus
	Ultima Gold XR	Pico-Fluor Plus
	Ultima Gold AB	
	Ultima Gold LLT	
	Opti-Fluor	

Table 2: Cocktail selection for medium ionic strength samples (based on the use of 10 mL of cocktail).

	Safer cocktail	Classical cocktail
Low sample volume (0 to 2.5 mL)	Ultima Gold	Insta-Gel Plus
	Ultima Gold XR	Pico-Fluor Plus
	Ultima Gold AB	Hionic-Fluor
	Ultima Gold LLT	
High sample volume (>2.5 mL)	Ultima Gold XR	Pico-Fluor Plus
		Hionic-Fluor

Table 3: Sample capacity of selected cocktails for various ionic strength buffers (sample capacities are for 10 mL cocktail at 20 °C).

Ionic strength	Ultima Gold XR	Hionic-Fluor	Pico-Fluor Plus	Ultima Gold	Ultima Gold MV	Opti-Fluor	Bio-Fluor Plus
0.5 M NaCl	9.0 mL	1.4 mL	3.0 mL	1.5 mL	1.25 mL	1.1 mL	1.2 mL
0.75 M NaCl	6.5 mL	2.25 mL	2.75 mL	0.75 mL	0.75 mL	0.75 mL	0.5 mL
1.0 M NaCl	5.5 mL	8.5 mL	2.3 mL	0.5 mL	0.5 mL	0.5 mL	0.25 mL

Buffers – High ionic strength

With high ionic strength buffers, the choice of cocktails is among Ultima Gold XR, Hionic-Fluor, and Pico-Fluor Plus. Certain high ionic strength samples can be accommodated in other cocktails, however the capacity is usually very low (less than 0.5 mL). The only other method of overcoming the problem of low sample acceptance of high ionic strength samples is to dilute the buffer sample with water and convert it into a medium strength buffer which simplifies cocktail selection. The sample capacity of selected cocktails for increasing ionic strength solutions is shown in Table 3.

Acids

This group includes commonly encountered mineral acids such as hydrochloric acid, nitric acid, sulphuric acid, perchloric acid, orthophosphoric acid, and hydrofluoric acid as well as some aqueous miscible organic acids such as acetic acid, formic acid, and trichloroacetic acid (TCA). Acids are commonly used as extractants,[4] pH modifiers, and solubilizers.[5] There are a number of potential problems associated with this particular sample group and these include quenching, reaction with cocktail components, and chemiluminescence. Strong mineral acids can also cause marked quenching effects, due primarily to interaction with the scintillators.

This can be overcome by using a cocktail which is known to be compatible with mineral acids or, preferably, by diluting the acid with water prior to the addition of the cocktail. Certain strong mineral acids will react with cocktail components causing both color development and changes in surfactant characteristics. For example, adding even small amounts of concentrated sulphuric acid to a cocktail will result in almost immediate color formation and eventual sulphonation of the surfactants (emulsifiers). This alteration to the surfactants will result in a change or loss of emulsifying properties and lead to phase instability. The color formation is due to sulphonation of minor impurities in the solvent and in addition to color, significant amounts of chemiluminescence may be produced. Another example involves adding small amounts of concentrated nitric acid which results in a yellow/brown color forming due to the dissociation of nitric acid and release of NO_2. This problem can be overcome by diluting the acid with water prior to adding it to the cocktail. With some cocktails, the addition of TCA can produce chemiluminescence. Although a rare occurrence, acid-induced chemiluminescence can be avoided by using a cocktail which is resistant to it such as Ultima Gold LLT. An overview of suitable cocktails for acids is shown in Table 4.

Alkalis

This group of samples includes bases such as sodium hydroxide, potassium hydroxide, and ammonium hydroxide. Alkaline samples are produced from applications involving pH modification, cell lysis, CO_2 trapping,[6] and solubilization. The major problem normally encountered is chemiluminescence and in general the amount of chemiluminescence is influenced by both the volume and concentration of alkali added. The standard method of avoiding this problem is to use a cocktail which is known to be resistant to chemiluminescence. Other methods of overcoming the problem include diluting the base with water to reduce the effect, allowing the chemiluminescence to decay in the dark before counting, and neutralizing the base with acid. Prolonged storage of cocktails with alkalis present is not recommended due to the potential for color formation. Where possible, counting should be performed within one or two days.

Other types

This final group covers other aqueous samples/mixtures which are occasionally used in LSC methods and assays. These aqueous mixtures are usually fairly specific for certain types of assays, *e.g.*, sucrose gradients in DNA and RNA separation, urea as a denaturing and reducing buffer, and imidazole as a biological buffer. Ultima Gold is the cocktail of choice for these sample types.

Table 4: Cocktail selection for acids (based on the use of 10 mL of cocktail).

Acid	Concentration	Safer cocktail	Classical cocktail
Mineral Acids	0 to 2M	Ultima Gold AB	Insta-Gel Plus
		Ultima Gold LLT	Pico-Fluor Plus
		Ultima Gold	Hionic-Fluor
	>2M	Ultima Gold AB	
		Ultima Gold LLT	
Trichloroacetic Acid	0 to 20%	Ultima Gold	Hionic-Fluor

Summary

A slightly expanded compilation of recommended cocktails for different sample types is shown in the Appendix in Table A (Safer Cocktails), Table B (Classical Cocktails), and Table C (Safer Cocktails for Acids). This may prove useful in providing a basic guide to cocktail selection. To further help with cocktail selection, sample capacities are presented to help with both the selection and suitability of a cocktail.

Conclusion

There are a variety of LSC cocktails, of both safer, high flash-point and of classical solvent types, which are suitable for use with the wide diversity of aqueous samples commonly encountered in LSC. If problems with sample/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

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5. LSC Counting Solutions (1996) *LSC Sample Preparation by Solubilization.* Revvity. CS-003.

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Appendix

Selection and suitability of a cocktail based on ionic strength

1. Determine the approximate ionic strength using the sample molarity:

$$\text{Mixture Molarity} = \frac{[(Va \times Ma) + (Vb \times Mb) + ...]}{(Va + Vb + ...)}$$

Where:
Va = Volume of solution A
Ma = Molarity of solution A
Vb = Volume of solution B
Mb = Molarity of solution B

Example: 10 mL of 0.2 M NaCl (solution A) added to 2 mL of 0.5 M KH_2PO_4 (solution B)

$$\text{Mixture Molarity} = \frac{[(10 \times 0.2) + (2 \times 0.5)]}{(10 + 2)}$$

= 0.25 M (A medium molarity sample)

2. Select appropriate cocktail based on ionic strength.

3. Test for sample/cocktail compatibility.

   3.1 Dispense 10.0 mL cocktail into a 20 mL glass vial. The use of a glass vial allows a clear view of the mixture.

   3.2 Add the desired sample volume, cap, and shake thoroughly.

   3.3 If the mixture is clear, proceed with the stability test.

   3.4 If the mixture is cloudy or hazy, try increasing the cocktail volume and/or decreasing the sample volume. If the mixture does not clear, select a cocktail which can accept higher ionic strength samples such as Ultima Gold XR.

   3.5 If the mixture separates into two distinct phases (like oil and water), or is milky, select a cocktail which can accept higher ionic strength samples.

4. Test for stability.

   Use a mixture which has passed the sample/cocktail compatibility test. Allow it to stand at the LSC counting temperature for a minimum of two hours or the proposed count time for the sample, if this is greater than two hours. If the mixture remains stable, successful counting will be possible.

5. Count.

   Note: Once the proper proportions and stability of the sample/cocktail mixture have been established, plastic vials can be considered for routine counting.

Table A: Performance of safer cocktails with various aqueous buffers at 20 °C (based on the use of 10 mL of cocktail). White area indicates no or very limited sample capacity.

Sample	ULTIMA Gold	ULTIMA Gold XR	ULTIMA Gold AB	ULTIMA Gold LLT	ULTIMA Gold MV	Opti-Fluor	Emulsifier-Safe
Deionized Water	3.2 mL	>10.0 mL	10.0 mL	>10.0 mL	2.0 mL	1.8 mL	3.2 mL
0.01 M PBS (pH 7.4)	6.5 mL	10.0 mL	8.5 mL	8.0 mL	4.0 mL	3.0 mL	3.0 mL
0.1 M PBS (pH 7.4)	4.0 mL	8.5 mL	<0.25 mL	<0.25 mL	3.0 mL	2.0 mL	0.6-1.2 mL
0.5 M PBS (pH 7.4)	0.5mL	1.25 mL			<0.5 mL		
0.05 M Tris-HCl (pH 7.4)	4.5 mL	10.0 mL	10.0 mL	9.0 mL	2.75 mL	2.7 mL	3.2 mL
0.15 M Sodium Chloride	6.5 mL	10.0 mL	7.5 mL	6.5 mL	5.0 mL	3.5 mL	3.1 mL
0.5 M Sodium Chloride	1.5 mL	9.0 mL	8.0 mL	6.0 mL	1.25 mL	1.1 mL	2.2 mL
1.0 M Sodium Chloride	0.5 mL	5.5 mL	4.5 mL	3.5 mL	0.5 mL	0.5 mL	1.4 mL
0.04 M NaH_2PO_4 (pH 7.4)	8.0 mL	10.0 mL	0.75-8.0 mL	0.75-7.0 mL	2.25 mL	4.25 mL	0.6-2.5 mL
0.1 M NaH_2PO_4 (pH 4.9)	10.0 mL	10.0 mL	8.0 mL	8.0 mL	7.0 mL	7.5 mL	2.0 mL
0.2 M NaH_2PO_4 (pH 4.9)	3.5 mL	10.0 mL	1.0-6.5 mL	1.0-5.0 mL	2.75 mL	3.0 mL	2.0 mL
0.25 M Amm. Acetate	3.25 mL	8.0 mL	5.0 mL	5.5 mL	3.0 mL	1.5 mL	2.0 mL
0.1 M Amm. Sulphate	3.25 mL	10.0 mL	1.0-7.0 mL	1.0-5.5 mL	2.25 mL	3.0 mL	2.0 mL
0.1 M Sodium Sulphate	4.25 mL	10.0 mL			3.25 mL	4.0 mL	1.75 mL
0.1 M HCl	6.5 mL	7.0 mL	10.0 mL	10.0 mL	4.5 mL	4.0 mL	2.7 mL
10% TCA	3.0 mL	7.0 mL	4.5 mL	4.0 mL	1.5 mL	2.5 mL	2.3 mL
20% TCA	2.0 mL	5.0 mL	3.0 mL	3.0 mL	0.5 mL	2.0 mL	1.5 mL
0.1 M NaOH	2.5 mL	10.0 mL	10.0 mL	7.5 mL	1.5 mL	5.0 mL	3.0 mL
1.0 M NaOH	3.0 mL	1.0 mL			1.75 mL	<0.25 mL	0.75 mL
0.1 M Imidazole (pH 7.4)	10.0 mL	10.0 mL	10.0 mL	10.0 mL	2.0 mL	4.5 mL	2.5 mL
8 M Urea	1.0 mL	2.5 mL	2.0 mL	3.5 mL	0.5 mL	0.5 mL	1.0 mL

Table B: Performance of classical cocktails with various aqueous buffers at 20 ˚C (based on the use of 10 mL of cocktail). White area indicates no or very limited sample capacity. *Clears only after extended agitation.

Sample	Insta-Gel Plus	Bio-Fluor Plus	Pico-Fluor Plus	Hionic-Fluor
Deionized Water	0-1.7 mL 2.9-10.0 mL	1.6 mL	2.3 mL	1.2 mL
0.01 M PBS	0.2-1.6 mL	2.9 mL	10.0 mL	1.4 mL
	3.1-10.0 mL			
0.1 M PBS	1.0-2.0 mL	<0.25 mL	6.4 mL	1.6 mL
0.5 M PBS			2.0 mL	7.0 mL
0.05 M Tris-HCl	0-1.8 mL	2.0 mL	3.0 mL	3.0 mL
	3.0-10.0 mL			
0.15 M Sodium Chloride	0-1.8 mL	4.0 mL	10.0 mL	1.1 mL
	4.9-10.0 mL			
0.5 M Sodium Chloride	0-2.1 mL	1.2 mL	3.0 mL	1.4 mL
	3.0-10.0 mL			
1.0 M Sodium Chloride	0.4-7.0 mL	<0.3 mL	2.3 mL	8.5 mL
0.04 M NaH_2PO_4	0.3-1.9 mL	2.0 mL	4.0 mL	1.75 mL
	3.0-10.0 mL			
0.1 M NaH_2PO_4 (pH 4.9)	0.6-2.0 mL	6.0 mL	10.0 mL	1.75 mL
0.2 M NaH_2PO_4 (pH 4.9)	1.0-2.0 mL	2.0 mL	7.1 mL	1.75 mL
	4.0-10.0 mL			
0.25 M Amm. Acetate	0-1.75 mL	1.5 mL	5.0 mL	1.75 mL
	3.5-10.0 mL			
0.1 M Amm. Sulphate	0.5-1.75 mL	2.5 mL	8.5 mL	1.75 mL
	3.5-10.0 mL			
0.1 M Sodium Sulphate		2.5 mL	8.5 mL	1.75 mL
0.1 M HCl	0-1.5 mL	1.8 mL	7.25 mL	1.3 mL
	3.0-10.0 mL			
10% TCA	2.1 mL			
	(No gel phase)	1.5 mL	4.0 mL	1.5 mL
20% TCA	3.75 mL			
	(No gel phase)	1.25 mL	3.25 mL	4.5 mL
0.1 M NaOH	0-2.0 mL	3.3 mL	2.5 mL	1.2 mL
	3.0-10.0 mL			
1.0 M NaOH		0.5 mL	4.5 mL	1.2 mL
0.1 M Imidazole (pH 7.4)	0-1.75 mL	3.0 mL	5.5 mL	1.75 mL
	3.0-10.0 mL			
8 M Urea	0-1.5 mL*	1.0 mL*	2.5 mL	1.0 mL

Table C: Recommended safer cocktails for mineral acids (based on the use of 10 mL of cocktail). White area indicates no or very limited sample capacity.

Sample	ULTIMA Gold	ULTIMA Gold XR	ULTIMA Gold AB	ULTIMA Gold LLT	Opti-Fluor	Emulsifier-Safe
0.1 M HCl	6.5 mL	7.0 mL	10.0 mL	10.0 mL	4.0 mL	2.7 mL
1.0 M HCl	0.5 mL	2.5 mL	5.5 mL	5.0 mL	0.5 mL	3.0 mL
2.0 M HCl		1.0 mL	2.25 mL	3.0 mL		4.5 mL
5.0 M HCl		<0.5 mL	2.0 mL	1.5 mL		0.5 mL
1.0 M HNO_3		2.5 mL	3.25 mL	3.5 mL	0.75 mL	3.5 mL
2.0 M HNO_3	0.5 mL	2.0 mL	2.25 mL	2.5 mL	0.75 mL	3.5 mL
3.0 M HNO_3		1.0 mL	2.0 mL	2.25 mL	0.5 mL	1.0 mL
1.0 M H_2SO_4		0.25 mL	6.5 mL	7.0 mL		2.0 mL
2.0 M H_2SO_4			4.0 mL	4.0 mL		2.75 mL
1.0 M $HClO_4$	2.0 mL	2.0 mL	2.25 mL	2.25 mL	1.5 mL	1.0 mL
2.0 M $HClO_4$	1.5 mL	1.5 mL	2.0 mL	2.5 mL	1.0 mL	0.75 mL
1.0 M H_3PO_4		1.5 mL	0.5-10.0 mL	0.5-10.0 mL	0.5-1.5 mL	3.0 mL
2.0 M H_3PO_4		0.5 mL	0.5-4.0 mL	0.5-6.0 mL	0.5-1.0 mL	3.0 mL

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