

## 液相閃爍計數 ( Liquid Scintillation Counting ) 相關資訊

### 樣本製備技術 ( Sample Preparation Techniques )

由於大部分放射線物質標定的生物性樣本 (如：全血，培養細胞，植物的根、莖、葉，等...) 無法直接進行計數定量的實驗，因此如何利用液相閃爍試劑 (Liquid Scintillation Cocktails, LSC cocktails) 將樣本製備成均勻穩定的液態樣本，成為在進行液相閃爍計數的過程中，非常關鍵重要的步驟之一。

附表一：樣本製備方式

Various Sample Preparation Techniques		
製備方式	敘述	樣本形式
Dissolving	直接將樣本溶於 LSC cocktails 中測試	有機樣本 (如：脂肪、固醇類等...)
Emulsifying	將樣本溶於含界面活性劑的 LSC cocktails 中乳化測試	鹽類或各式酸鹼緩衝液
Suspending	將不溶解的樣本懸浮於一穩定的膠質中	懸浮於 Insta-Gel Plus 中的土質樣本
Extracting or Eluting	將固定層中的標定物質溶出於 LSC cocktails 或 solubilizers 中測試	濾紙或膠體類樣本 (如：TLC-scrappings、Paper-chromatograms、Polyacrylamide gel slices、Cellulose nitrate filters)
Solubilizing	以鹼性的 Soluene-350 試劑溶解組織樣本	細胞、組織或排泄物
Wet oxidation	將樣本溶於含強酸及過氧化氫的 solubilizer 中進行分解及測試	以硝酸或是過氧酸中的過氧化氫分解植物樣本
Combustion	利用 Carbo-Sorb E, Soluene-350 或 Hyamide Hydroxide 吸收 $^{14}\text{CO}_2$ ; 利用 Monophase S 測試 $^3\text{H}_2\text{O}$ 樣本	$^{14}\text{CO}_2$ 或 $^3\text{H}_2\text{O}$ 樣本

## 樣本製備常見問題

在利用 LSC cocktails 製備好樣本後，在上機計數以前需先確認 sample/cocktail mixture 是否已確實混和均勻，否則會導致計數結果的不正確性。附表中為常見的樣本製備不完全，或使用不適當的 cocktails 所導致的問題。

Problem	Cause	Remedy
不穩定的計數率 (CPM 值不斷減少)	<ol style="list-style-type: none"> <li>1. 樣本與 cocktails 未混合均勻分成兩層</li> <li>2. 放射性標定樣本沈澱</li> <li>3. 化學冷光</li> </ol>	<ol style="list-style-type: none"> <li>1. 增加 cocktails 的使用量或使用高樣本承載容積 (high sample capacity) 的 cocktails</li> <li>2. 改用能夠容許樣本含有高鹽的 cocktails</li> <li>3. 使用 Hionic-Fluor 或是 Ultima Gold</li> </ol>
不穩定的計數率 (CPM 值不斷增加)	<ol style="list-style-type: none"> <li>1. 樣品與 cocktails 混合不夠完全</li> <li>2. 樣本放置過久，導致樣本與 cocktails 分成兩層</li> <li>3. 樣本未充分從固定層中沖提出來</li> </ol>	<ol style="list-style-type: none"> <li>1. 充分混和樣品與 cocktails</li> <li>2. 選擇適合的 cocktails 使用</li> <li>3. 以下列溶劑充分的將樣本從固定層中沖提出來               <ol style="list-style-type: none"> <li>A. 水，酸或鹼溶劑</li> <li>B. Soluene-350</li> </ol> </li> <li>4. 以 Filter-Count 溶解含濾紙的樣本</li> </ol>
計數效率降低	<ol style="list-style-type: none"> <li>1. 樣本本身帶有高含量的色素因子</li> <li>2. 樣本帶有高含量的化學物質</li> </ol>	<ol style="list-style-type: none"> <li>1. 在加入 cocktails 前，先將樣品脫色處理，如使用 hydrogen peroxide (可能會因為形成氫氣損失 <math>^3\text{H}</math>)</li> <li>2. 增加 cocktails 的體積或改用能夠抵抗抑制作用的 cocktails，如 Ultima Gold</li> </ol>

## 樣品製備流程簡介

下列表格主要是將幾種生物性樣本的製備處理流程簡單加以分類敘述，詳細的樣本處理流程則可參照 PerkinElmer 網站 (<http://las.perkinelmer.com/>) 各 LSC 產品的介紹說明。

Sample Biological Fluids	Procedure Steps					Recommended Sample Size	Expected <sup>3</sup> H Efficiency
	#1	#2	3	#4	#5		
Blood	Add to 0.1-0.4 mL blood 1 mL mixture of Soluene-350:IPA (1.1)	Stand 2 hrs at 60°C	Add 0.2-0.5 mL 30% H <sub>2</sub> O <sub>2</sub> dropwise with swirling	Stand 15-30 min at ambient. Cap tightly. Stand 30 min at 60°C	Add 10-15 mL Hionic-Fluor	Up to 0.4 mL blood	20-30%
	Add to 0.1-0.5 mL blood 1 mL SOLVABLE	Stand 1 hr at 60°C	Add 0.1 mL 0.1 M EDTA-Na <sub>2</sub> solution. Add 0.3-0.5 mL 30% H <sub>2</sub> O <sub>2</sub> in aliquots.	Stand 15-30 min at ambient. Cap tightly. Stand 30 min at 60°C	Add 10-15 mL Ultima Gold	Up to 0.5 mL blood	25-35%
Plasma or Serum	Add up to 1 mL plasma or serum to 10-15 mL of Ultima Gold	Shake until clear				Up to 1 mL	30-40%
Red Blood Cells (RBC)	0.2 mL RBC suspension + 1 mL Soluene-350: IPA mixture (1:1)	Stand 1 hr at 60°C	Add 0.2-0.5 mL 30% H <sub>2</sub> O <sub>2</sub> dropwise with swirling	Stand 15-30 min at ambient. Cap tightly. Stand 30 min at 60°C	Add 10-15 mL Hionic-Fluor	Up to 0.2 mL RBC suspension	20-30%
	0.2 mL RBC suspension + 1 mL SOLVABLE	Stand 1 hr at 60°C	Add 0.1 mL 0.1 M EDTA-Na <sub>2</sub> solution. Add 0.3-0.5 mL 30% H <sub>2</sub> O <sub>2</sub> in aliquots	Stand 15-30 min at ambient. Cap tightly. Stand 30 min at 60°C	Add 10-15 mL Ultima Gold	Up to 0.2 mL RBC suspension	25-35%
Urine	Add up to 8 mL of urine to 12 mL of Ultima Gold LLT and shake vigorously					Up to 8 mL	25-35%
Phosphate Buffered Saline (PBS)	Add up to 8 mL of 0.01 M PBS to 10 mL of Ultima Gold LLT	or	Add up to 4 mL of 0.01 M PBS to 10 mL Ultima Gold	or	Add up to 10 mL of 0.01 M PBS to 10 mL Ultima Gold XR	Up to 10 mL	30-40%
Aqueous Proteinaceous Sample	Add 0.2 mL of sample to 1 mL Soluene-350	Swirl until clear	Add 10 mL Hionic-Fluor			Up to 0.2 mL	35-40%
	Add 0.2 mL of sample to 1 mL SOLVABLE	Swirl until clear or heat 30 min at 50°C	Add 10 mL Ultima Gold			Up to 0.2 mL	35-45%
Sucrose Solutions	Add between 3 and 7 mL of 20-40% (w/v) sucrose to 10 mL Ultima Gold XR	or	Add between 5 and 10 mL of 30-60% (w/v) sucrose to 10 mL Hionic-Fluor			Up to 10 mL	30-40%
Inulin Containing Fluid	Add 50 µL of inulin sample to 0.5 mL of Soluene-350 and swirl	Add 10 mL of Hionic-Fluor				Up to 50 µL	40-50%
Trichloroacetic Acid (TCA) supernatant	Add up to 3 mL of up to 20% TCA supernatant to 10 mL of Ultima Gold LLT	For concentrations over 20% use Hionic-Fluor				Up to 3 mL	25-40%

Sample Biological Tissues	Procedure Steps					Recommended Sample Size	Expected <sup>3</sup> H Efficiency
	#1	#2	#3	#4	#5		
Homogenate	Add 0.2 mL of up to 10% tissue homogenate (in either water or 70% ethanol) to 3 mL of water	Add 10 mL of Insta-Gel Plus	Shake vigorously		Note: Homogenates can also be prepared as for coarse-ground tissue	Up to 0.2 mL of 10% tissue homogenate	30-40%
Coarse-Ground Tissue	Add 150 mg coarse-ground tissue to 2 mL Soluene-350 and swirl	Stand for 3-5 hr at 60°C	Add 10 mL Hionic-Fluor			Up to 1.0 mL of 10% tissue homogenate. Up to 150 mg coarse-ground tissue.	35-40%
	Add 150 mg coarse-ground tissue to 2 mL SOLVABLE and swirl	Stand for 3-5 hr at 60°C	Add 10 mL Ultima Gold			Up to 1.0 mL of 10% tissue homogenate. Up to 150 mg coarse-ground tissue.	35-45%
Bacteria and Cells	Add 1 mL of 8:2 Soluene-350: water to 5-7 mg of bacteria or cells	Stand 2-4 hr at 60°C	Add 10 mL Hionic-Fluor			5-7 mg of bacteria or cells	20-30%
	Add 1 mL of SOLVABLE to 5-7 mg of bacteria or cells	Stand 2-4 hr at 60°C	Add 10 mL Ultima Gold			5-7 mg of bacteria or cells	35-45%
Organs	Add 1 mL Soluene-350 per:	Stand 2-4 hr at 60°C	Add 10 mL Hionic-Fluor			"See Steps 1, 2 and 3".	17-40%
	Arteries: 30-100 mg Brain: 50-150 mg Cartilage: 20-55 mg Cornea: 40-160 mg Heart: 50-100 mg	Intestine: 80-100 mg Kidney: 50-100 mg Liver: 50-100 mg Muscle: 100-200 mg Nerve cells: 50-100 mg	Pancreas: 50-110 mg Spleen: 50-140 mg Stomach: 50-100 mg Sinew: 50-150 mg				
	Add 1 mL SOLVABLE per:	Stand 2-4 hr at 60°C	Add 10 mL Ultima Gold			"See Steps 1, 2 and 3."	30-45%
	Arteries: 30-100 mg Brain: 50-150 mg Cartilage: 20-55 mg Cornea: 40-160 mg Heart: 50-100 mg	Intestine: 80-100 mg Kidney: 50-100 mg Liver: 50-100 mg Muscle: 50-100 mg Nerve cells: 50-100 mg	Pancreas: 50-110 mg Spleen: 50-140 mg Stomach: 50-100 mg Sinew: 50-150 mg				

Sample Biological Tissues	Procedure Steps					Recommended Sample Size	Expected <sup>3</sup> H <sup>1</sup> Efficiency
	#1	#2	#3	#4	#5		
Feces	Add 0.1 mL water to 20 mg feces (dried), rehydrate for 30 min	Add 1 mL Soluene-350	Stand 1-2 hr at 50°C. Add 1 mL IPA and mix. Stand 2 hr at 50°C.	Add 0.2 mL of 30% H <sub>2</sub> O <sub>2</sub> dropwise with swirling	Stand 10 min ambient; add 10 mL Hionic-Fluor	Up to 20 mg feces (dried)	25-35%
	Add 0.1 mL water to 20 mg feces (dried), rehydrate for 30 min	Add 1 mL SOLVABLE	Stand 1-2 hr at 50°C. Add 1 mL IPA and mix. Stand 2 hr at 50°C.	Add 0.2 mL of 30% H <sub>2</sub> O <sub>2</sub> dropwise with swirling	Stand 10 min ambient; add 10mL Ultima Gold	Up to 20 mg feces (dried)	30-40%
TLC <sup>3</sup> -Scrapings	Add water soluble sample on TLC silica to 1 mL of H <sub>2</sub> O	Stand for 3-5 hr at 40°C, add 8-10 mL of InstaGel Plus	Note: If samples are not water soluble add 1 mL of Soluene-350 instead of H <sub>2</sub> O	Stand 3-5 hr at 40°C add 10 mL of Hionic-Fluor			30-40%
Polyacrylamide Gel Slices (PAGE)	Add 1-2 mm gel slice to 0.5 mL Soluene-350	Stand for 3 hr at 50°C	Add 10mL Hionic-Fluor			1-2 mm gel slice	45-50%
	Add 1-2 mm gel slice to 0.5 mL SOLVABLE	Stand for 3 hr at 50°C	Add 10 mL Ultima Gold			1-2 mm gel slice	50-50%
TCA Precipitates	Moisten 100 mg of dried TCA precipitate (proteinaceous) with 0.1-0.2 mL water	Rehydrate for 30 min	Add 1 mL Soluene-350 and stand 30 min at ambient	Add 10 mL Hionic-Fluor		Up to 100 mg	35-40%
	Moisten 100 mg of dried TCA precipitate (proteinaceous) with 0.1-0.2 mL water	Rehydrate for 30 min	Add 1 mL SOLVABLE and stand 30 min at ambient	Add 10 mL Ultima Gold		Up to 100 mg	40-50%
Filters	(Cellulose acetate only) Place filter on bottom of vial. Rehydrate with 0.1-0.2 mL H <sub>2</sub> O	Add 0.5-1.0 mL Soluene-350; stand for 30 min at ambient.	Add 10 mL Hionic-Fluor				50-55%
	(Cellulose acetate only) Place filter on bottom of vial. Drying wet filters is not required.	Add 5-10 mL Filter-Count. Shake several times until filter is dissolved and count.					35-45%

**Abbreviations:**

<sup>1</sup> Tritium counting efficiency was determined on a PerkinElmer Tri-Carb Model 3100TR with 65% efficiency.

<sup>2</sup> IPA = Isopropanol

<sup>3</sup> TLC = Thin layer chromatogram

*Note: The PerkinElmer 307 Sample Oxidizer can easily be used to prepare up to 1-2 grams of any of the listed samples.*

## LSC Cocktail 比較表

可參照下表選擇適當的 LSC cocktail

	COCKTAIL	TYPE OF SOLVENT	FLASH-POINT °C/Tag CC.	COUNTING EFFICIENCY* -%			SAMPLE LOAD CAPACITY <sup>2</sup> -mL					
				No Sample	With 10% Water	With 10% Solubilizer	Water	SAMPLE LOAD CAPACITY <sup>2</sup> -mL				
								0-0.05 M	0.05-0.2 M	0.2-0.5 M	0.5-1.0 M	Over 1.0 M
COCKTAILS FOR AQUEOUS SAMPLES	ULTIMA GOLD	DIPN	~150	56	52	49 <sup>3</sup>	3.2	3.0-6.0	3.0-5.0	2.0-4.0	1.0-4.0	0-3.0
	ULTIMA GOLD XR	DIPN	~150	50	46	N.A.	10.0	8.0-10.0	8.0-10.0	5.0-8.0	3.0-7.0	0-5.0
	ULTIMA GOLD LLT	DIPN	~140	52	46	N.A.	12.0	Optimized for all water types				
	ULTIMA GOLD MV	DIPN	~110	57	55	N.A.	1.0	1.0-3.0	2.0-4.0	2.0-4.0	0-2.0	*
	ULTIMA GOLD AB	DIPN	~140	52	46	N.A.	10.0	Optimized for 1-2 M mineral acids				
	HI-SAFE 2	DIPN	~150	56	52	49 <sup>3</sup>	3.2	3.0-6.0	3.0-5.0	2.0-4.0	1.0-4.0	0-3.0
	HI-SAFE 3	DIPN	~150	50	46	N.A.	10.0	8.0-10.0	8.0-10.0	5.0-8.0	3.0-7.0	0-5.0
	OPT-FLUOR	Linear Alkylbenzene	~150	44	40	N.A.	1.5	1.5-2.0	1.5-2.5	0.5-1.0	*	-
	EMULSIFIER-SAFE	Linear Alkylbenzene	~150	43	39	N.A.	1.5	1.5	1.5	1.0-1.5	0.5-1.0	-
	INSTA-GEL PLUS	Pseudocumene	48-50	56	48	N.A.	1.8 & 3-10	1.8 & 3-10	1.8 & 3-10	0.5-1.0 & 3-10	0.5-1.5	0.1-1.5
PICO-FLUOR 15	Pseudocumene	48-50	57	53	N.A.	1.5	1.5-2.0	1.5-2.5	0.5-1.0	-	-	
PICO-FLUOR 40	Pseudocumene	48-50	51	45	N.A.	3.0	1.5-2.0	5.0-10.0	2.0-2.4	1.0-2.0	0.5-1.0	
FILTER-COUNT	Pseudocumene	48-50	57	53	N.A.	1.0	*	*	*	*	*	
HIONIC-FLUOR	Pseudocumene	48-50	51	45	48	1.0	*	*	1.0-1.5	1.5-2.5	1.5-4.0	
ORGANIC SAMPLES	ULTIMA GOLD F	DIPN	~150	65	N.A.	N.A.	For organic samples and dried filter membranes only.					
	SCINT HI-SAFE	DIPN	~150	65	N.A.	N.A.						
	OPT-FLUOR O	Linear Alkylbenzene	~150	59	N.A.	N.A.						
	INSTA-FLUOR PLUS	Pseudocumene	48-50	65	N.A.	57	For organic samples only.					

<sup>1</sup> Typical counting efficiencies determined on a Perkin Elmer Tri-Carb Model 3100TR/LL (preset 3H region, 0-18.0 keV).

<sup>2</sup> Typical maximum sample volume (ml) per 10 ml cocktail at 20°C

<sup>3</sup> Ultima Gold with tissue solubilizers; preferably counted within 24 hours.

\* = limited capacity  
- = no capacity

## Flow Cocktail selection guide

