APPLICATION NOTE

Liquid Scintillation

Highlights

- Use and suggestion of chemical agents for radio-carbon dioxide trapping
- Recommended cocktail choices for counting trapped radio-carbon

Radio-Carbon Dioxide ($^{14}CO_2$) Trapping and Counting

Introduction

The PerkinElmer Sample Oxidizer is perfectly designed to trap $^{14}CO_2$ originating from the combustion of large amounts of samples containing ^{14}C. However discrete gaseous $^{14}CO_2$ samples not originating from combustion require a completely different approach for the trapping of the gas and subsequent liquid scintillation counting (LSC). $^{14}CO_2$ gas samples originate from a variety of sources, including:

1. $^{14}CO_2$ in expired breath
2. $^{14}CO_2$ expired by plants
3. $^{14}CO_2$ expulsion from blood
4. $^{14}CO_2$ released in enzymatic studies

There are a large number of potentially useful reagents available for trapping carbon dioxide, including the following:

1. Sodium hydroxide (0.1 - 1.0 M)
2. Potassium hydroxide (0.1 - 1.0 M)
3. Hyamine Hydroxide® in methanol (1.0 M)
4. Ethanolamine®
5. Carbo-Sorb E

Table 1 lists some basic information which will be helpful in selecting the best trapping reagent for a particular application.

Table 1. Trapping capacity of suitable reagents for carbon dioxide.

	mmol CO_2 per mL	mL required for 1 mmol CO_2	mL required for 5 mmol CO_2	mL required for 10 mmol CO_2	Flash-Point (°C)
0.1M Sodium/Potassium hydroxide	0.05	20.00	—	—	—
1.0M Sodium/Potassium hydroxide	0.50	2.00	10.00	—	—
1.0M Hyamine Hydroxide® in methanol	0.50	2.00	10.00		11 °C
Ethanolamine	8.10	0.12	0.62	1.23	93 °C
Carbo-Sorb E	4.80	0.21	1.04	2.08	27 °C

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Having selected a suitable carbon dioxide trapping reagent for the application, the next step is to ensure that a compatible liquid scintillation cocktail is chosen. Therefore, each reagent will be considered separately.

1-2. Sodium/Potassium hydroxide

Sodium hydroxide absorbs/traps CO_2 by a reaction which produces a sodium carbonate solution. Potassium hydroxide performs in a similar way by forming potassium carbonate.

Recommended LSC Cocktails

1. 10 mL of Emulsifier-Safe™* will accept up to 2 mL of 0.1 M sodium hydroxide/CO_2.
2. 10 mL of Opti-Fluor®* will accept up to 5 mL of 0.1 M sodium hydroxide/CO_2.
3. 10 mL of Hionic-Fluor™ will accept up to 5 mL of 1.0 M sodium hydroxide fully saturated with CO_2.
4. 10 mL of Ultima-Flo™ AF* will accept 10 mL of 0.5 M NaOH/CO_2 or 5 mL of 1.0 M NaOH/CO_2.

* Safer cocktails: High flash-point cocktails.

Notes:

1. Hionic-Fluor is suitable for use with hydroxide/carbonate solutions due to its sample capacity for concentrated solutions and alkaline pH. Ideally the final pH should be above 9 to avoid liberation of trapped CO_2.
2. Cocktails containing mixed surfactant systems, such as Ultima Gold™ or Ultima Gold XR can be used, however counting should be performed the same day as these cocktails have the potential for slow release of CO_2 on prolonged storage (i.e. characterized by dropping CPM levels).

3. Hyamine Hydroxide® in methanol

Chemically, Hyamine Hydroxide® performs similarly to sodium and potassium hydroxide in that it forms hyamine carbonate on reaction with CO_2.

Recommended LSC Cocktails

1. 10 mL of Insta-Fluor™ will accept up to 7.5 mL of Hyamine Hydroxide® saturated with carbon dioxide.
2. 10 mL of Emulsifier-Safe™ will accept up to 3 mL of Hyamine Hydroxide® saturated with carbon dioxide providing a safer system due to the high flash-point of this LSC cocktail.

Note:

Foaming of Hyamine Hydroxide® used to absorb carbon dioxide expelled in rat breath has been reported. This can be overcome by the addition of one drop of silicone antifoam per 10 mL of Hyamine Hydroxide®.

The chemiluminescence resistance of both these cocktails is shown in Table 2, which clearly demonstrates their suitabilities for use with this reagent system.

Table 2. Chemiluminescence resistance of LSC cocktails to Hyamine Hydroxide®/CO_2.

	Up to 3,0 mL Hyamine Hydroxide®/CO_2 in 10 mL Emulsifier-Safe		
Counting window	0 - 156	2 - 156	4 - 156
CPM after one minute	53	32	24
CPM after five minutes	39	27	22
CPM after one hour	36	31	25

	Up to 7.5 mL Hyamine Hydroxide®/CO_2 in 10 mL Insta-Fluor		
Counting window	0 - 156	2 - 156	4 - 156
CPM after one minute	35	28	22
CPM after five minutes	34	30	27
CPM after one hour	24	23	18

1) All counting in a PerkinElmer Tri-Carb® 1900@ 20 °C.
2) All samples fully saturated with carbon dioxide.
3) All counting in high performance glass vials.

Table 3. Suitable LSC solutions and capacity for ethanolamine/CO_2.

COCKTAIL	Cocktail Volume (mL)	Methyl Cellosolve Volume (mL)	Ethanolamine Volume (mL)	CO_2 Trapping Capacity (mmol)
Hionic-Fluor	10.0	4.0	1.0	8.1
Hionic-Fluor	10.0	6.5	2.0	16.2
Hionic-Fluor	10.0	8.5	3.0	24.3

4. Ethanolamine

Ethanolamine reacts with CO_2 in a rather different way than previously discussed reagents in that a carbamate is formed as opposed to a carbonate. The main difference between these two species is that a carbamate is more stable under slightly acidic conditions whereas a carbonate reacts rapidly with acids to release carbon dioxide.

Ethanolamine/CO_2 is notoriously difficult to incorporate into LSC cocktails and consequently the recommended LSC solutions may seem a little unusual.

Recommended LSC Cocktails

The main system known which is capable of accepting this unusual reagent is shown in Table 3. The recommended LSC cocktail requires the use of a co-solvent e.g. methyl cellosolve to facilitate the take up of the ethanolamine/CO_2.

5. Carbo-Sorb E

This amine reacts with CO_2 to form a carbamate as well. This reagent was developed to work in the PerkinElmer Sample Oxidizer. It is however, possible to use this reagent as carbon dioxide trapping agent outside of the Oxidizer. As with the Oxidizer, the recommended cocktail is Permafluor® E+. When using Carbo-Sorb E with Permafluor E+ , the following conditions are recommended:

For ratios of Carbo-Sorb E to Permafluor E+ from 1:10 up to 1:1, maximum saturation of carbon dioxide is possible with no phase separation of the resulting carbamate.

Note:

The use of Carbo-Sorb E is not recommended in enzymatic, plant or human studies due to the corrosive nature of the volatile amine present.

Summary

The information presented in the previous sections (1-5) of this publication are condensed into a quick reference table (Table 4). This may prove particularly useful when the total trapping capacity per standard 20 mL LSC vial is required.

Table 4. Reference table for CO_2 trapping and LS counting.

CO_2 Absorber	mmol CO_2 per mL	mL for 1 mmol CO_2	mL for 5 mmol CO_2	mL for 10 mmol CO_2	LSC Cocktail	Cocktail Volume	mL of Absorber	Max. CO_2 Capacity (mmol)
0.1 M Sodium & Potassium Hydroxide	0.05	20.00	—	—	Emulsifier-Safe	15.0 mL 14.0 mL	3.00 7.00	0.15 0.35
0.5 M Sodium & Potassium Hydroxide	0.25	4.00	—	—	Ultima-Flo AF	10.0 mL	10.00	2.50
1.0 M Sodium & Potassium Hydroxide	0.50 0.50	2.00 2.00	10.00 10.00	—	Hionic-Fluor Ultima-Flo AF	14.0 mL 14.0 mL	7.00 7.00	3.50 3.50
1.0 M Hyamine Hydroxide® in Methanol	0.50	2.00	10.00	—	Emulsifier-Safe Insta-Fluor	15.0 mL 12.0 mL	4.50 9.00	2.25 4.50
Ethanolamine	8.10	0.12	0.62	1.23	Hionic-Fluor + Methyl Cellosolve	10.0 mL 8.5 mL	3.00	24.30
Carbo-Sorb E	4.80	0.21	1.04	2.08	Permafluor E+	10.0 mL	10.00	48.00

Conclusion

There are a variety of PerkinElmer LSC cocktails, both high flash-point and classical types, which are suitable for $^{14}CO_2$ absorption work regardless of the trapping agent used. If problems with trapping and counting persist, or an alternative trapping agent not covered in this publication is used, please call your local PerkinElmer representative for further applications support.

Recommended Literature

1. Accurate Determination of $^{14}CO_2$ by Expulsion from Blood. Kaczmar, B.U. and Manet R. *Appl. Radiat. Isot.* Vol **38** No. 7, pp 577-578, 1987.
2. The Use of CO_2 Absorbers for the Determination of Specific ^{14}C Activities. R.M. Qureshi, Peter Fritz and R.J. Dsimmie, *Int. J. Appl. Radiat. Isot*. vol **36** No.2, pp 165-170, 1985.
3. ^{14}C Dating of Hydrological Samples Using Simple Procedures. Riffat M. Qureshi and Peter Fritz. *Int. J. Appl. Radiat. Isot*. vol **36** No. 10 p 825,1985.
4. A Device for the Liberation and Determination of $^{14}CO_2$. Schadewaldt et. al. *Analytical Biochemistry Vol* **132**, pp 400-404, 1983.
5. A Method of Counting ^{14}C as $CaCO_3$ in a Liquid Scintillator with Improved Precision. Pfeiffer K., Rank, D. and Tschurlovits. *Int. J. of App. Radiat. Isot*. vol **32**, pp 665-667, 1981.
6. Improved Technique for Accurate and Convenient Assay of Biological Reactions Liberating $^{14}CO_2$. Sissons, C.H. *Analytical Biochemistry* Vol **70**, pp 454-462, 1976.

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