# LSC in practice: radio-carbon dioxide ($^{14}CO_2$) trapping and counting.

## Introduction

The 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.

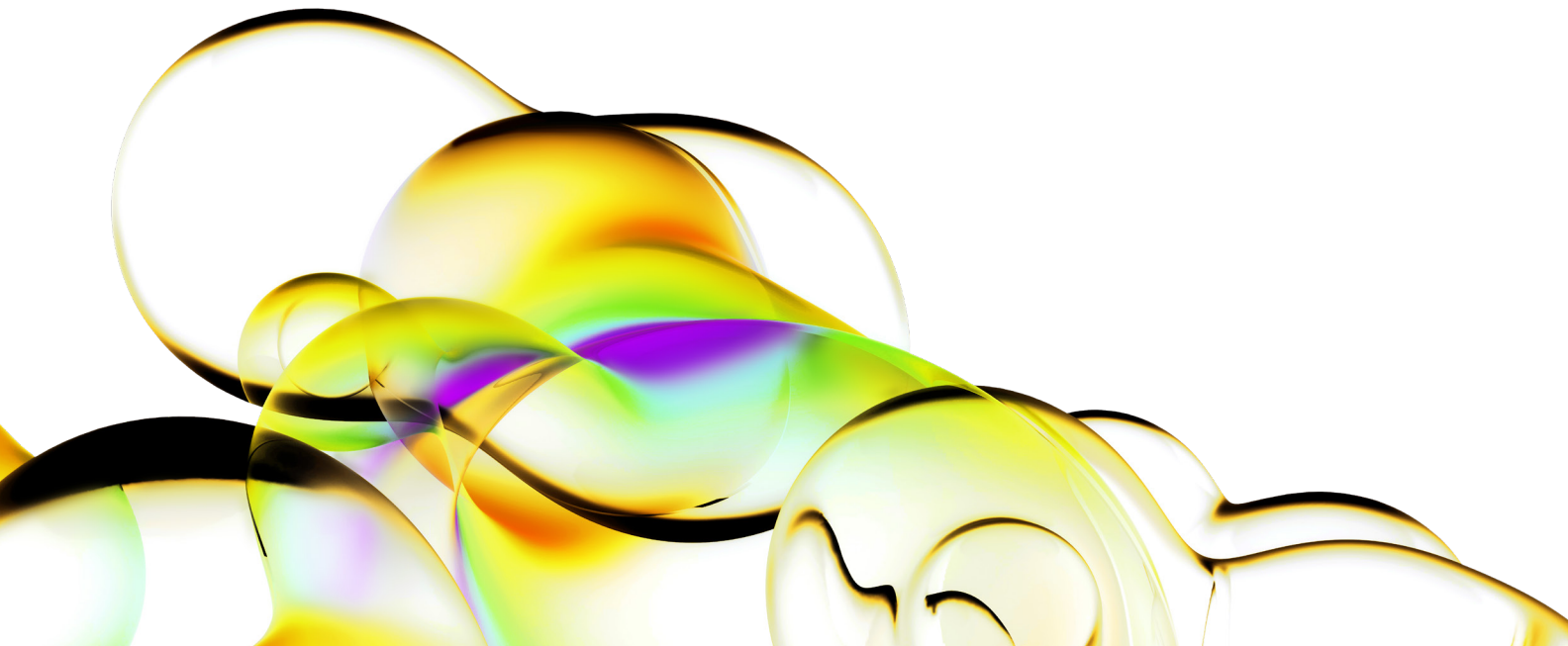

Table 1: Trapping capacity of suitable reagents for carbon dioxide.

| | mM $CO_2$ per mL | mL required for 10 mM $CO_2$ | mL required for 5 mM $CO_2$ | mL required for 1 mM $CO_2$ | 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 |

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 Hionic-Fluor™ will accept up to 5 mL of 1.0 M sodium hydroxide fully saturated with $CO_2$.

* High flash-point cocktail.

### 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™ Plus 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 Tri-Carb 1900@ 20 °C.

2. All samples fully saturated with carbon dioxide.

3. All counting in high performance glass vials.

## 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/$CO_2$.

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

| COCKTAIL | Cocktail Volume (mL) | Methyl Cellosolve Volume (mL) | Ethanolamine Volume (mL) | mM $CO_2$ Trapping Capacity |
|---|---|---|---|---|
| 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 |

## 5. Carbo-Sorb E

These amines react with $CO_2$ to form a carbamate as well. Both reagents were developed to work in the Revvity Sample Oxidizer. It is however, possible to use these reagents as carbon dioxide trapping agents outside of the Oxidizer. As with the Oxidizer, the recommended cocktail is Permafluor™ E+. When using 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 (1-6) 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.

| CO2 absorber | mM CO2 per mL | mL for 1 mM $CO_2$ | 5mM $CO_2$ mL for | 10 mM $CO_2$ mL for | LSC cocktail | Cocktail volume | mL of absorber | Max. $CO_2$ capacity (mM) |
|---|---|---|---|---|---|---|---|---|
| 0.1 M sodium & potassium hydroxide | 0.05 | 20.00 | — | — | Emulsifier-Safe | 15.0 mL<br>14.0 mL | 3.00<br>7.00 | 0.15<br>0.35 |
| 1.0 M sodium & potassium hydroxide | 0.50 | 2.00 | 10.00 | — | Hionic-Fluor | 14.0 mL | 7.00 | 3.50 |
| 1.0 M hyamine hydroxide in methanol | 0.50 | 2.00 | 10.00 | — | Emulsifier-Safe<br>Insta-Fluor | 15.0 mL<br>12.0 mL | 4.50<br>9.00 | 2.25<br>4.50 |
| Ethanolamine | 8.10 | 0.12 | 0.62 | 1.23 | Hionic-Fluor + methyl cellosolve | 10.0 mL<br>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 Revvity 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 Revvity 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.