

Use and preparation of quench curves in liquid scintillation counting.

Introduction

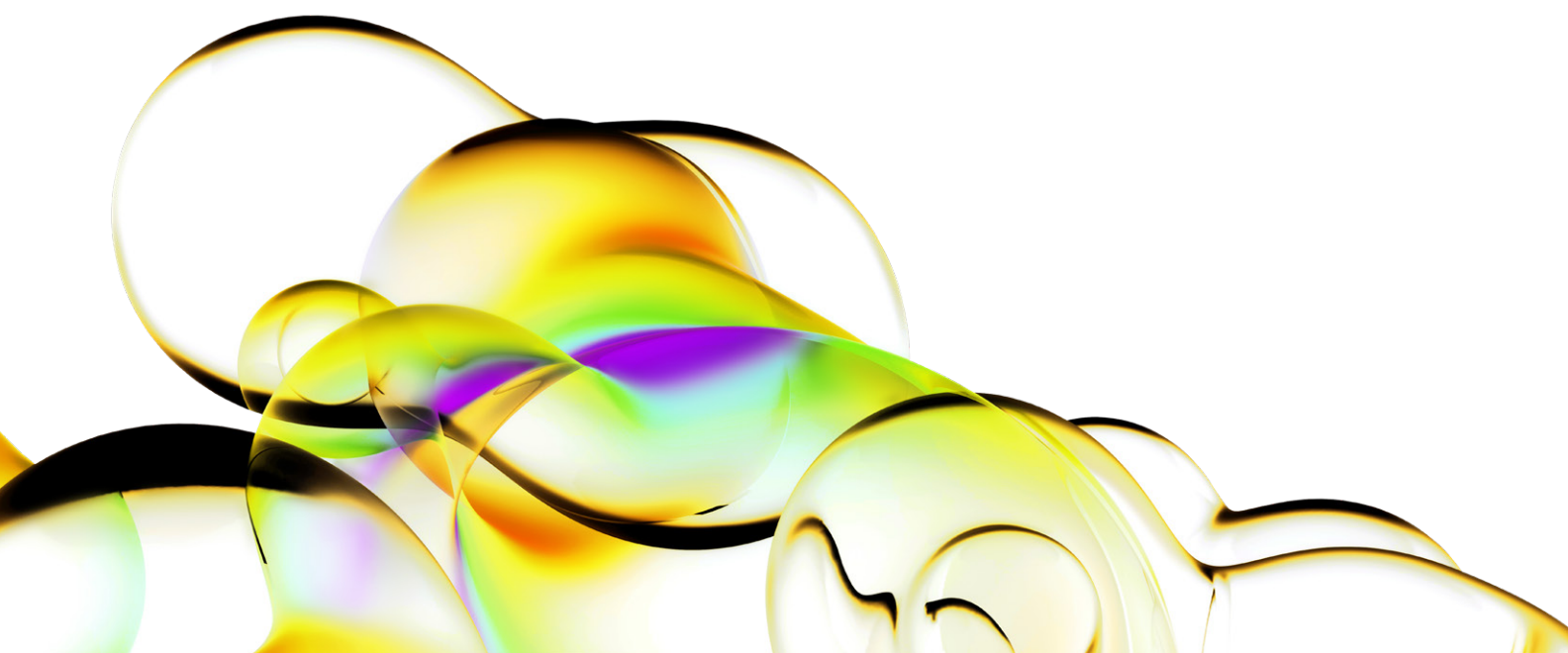
The liquid scintillation process is the conversion of the energy of a radioactive decay event into photons of light. Photomultiplier tubes (PMT's) are used to detect and convert the photons into electrical pulses. Both the sample and the scintillator are dissolved in an aromatic solvent, which allows energy to be transferred. Any factor, which reduces the efficiency of the energy transfer or causes the absorption of photons (light), results in quenching in the sample. There are two main types of quench: chemical quench^{1,2} and color quench^{1,3,4}.

Chemical quench

Chemical quench occurs during the transfer of energy from the solvent to the scintillator. Any chemical species that is electronegative (electron capturing) will affect the energy transfer process by capturing or stealing the π electrons associated with the aromatic solvent and thus reduce the availability of π electrons necessary for efficient energy transfer.

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Color quench

Color quench is an attenuation of the photons of light. The photons produced are absorbed or scattered by the color in the solution, resulting in reduced light output available for measurement by the PMT's. The steps in the energy transfer process affected by chemical and color quenching are indicated in Figure 1.

The collective effect of quench is a reduction in the number of photons produced and, therefore, detected CPM (counts per minute). Counting efficiency is affected by the degree of quenching in the sample.

To determine absolute sample activity in DPM (disintegrations per minute or absolute activity), it is necessary to measure the level of quench of the samples first, then make the corrections for the measured reduction in counting efficiencies

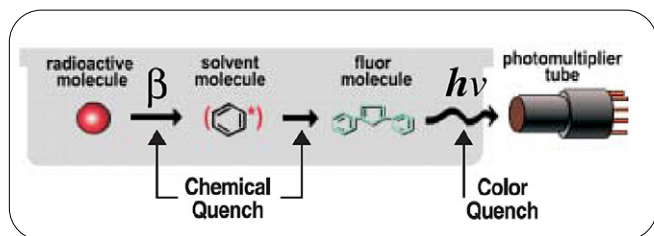


Figure 1. Quenching in the Energy Transfer Process

Measurement of quench

It is possible to measure quench accurately via high-resolution spectral analysis. Quenching manifests itself by a shifting of the energy spectrum toward lower energy channels in the Multichannel Analyzer (MCA). On Revvity's Tri-Carb® series LSA's, there are two methods of spectral analysis for measuring quench.

The first method is the Spectral Index of the Sample (SIS) which uses the sample isotope spectrum to monitor the quench of the solution. The SIS value decreases as quench increases, reflecting the shift of the spectrum to lower energy. The second method used to measure quench is the transformed Spectral Index of the External Standard (t-SIE) which is calculated from the Compton spectrum induced in the scintillation cocktail by an external ¹³³Ba gamma source.

The source is positioned under the sample vial, causing a Compton spectrum to be produced in the cocktail solution. From a mathematical transformation of this spectrum, the t-SIE value is determined, and t-SIE is a relative value,

on a scale from 0 (most quenched) to 1000 (unquenched). The calculated t-SIE value is adjusted to 1000 when the instrument is calibrated. Like SIS, t-SIE decreases as quench increases. Both SIS and t-SIE are used as Quench Indicating Parameters (QIP's). t-SIE is independent of the sample isotope and of the activity in the vial and has a large dynamic range. This makes it a very reproducible means of tracking the quench of the cocktail. SIS uses the sample isotope spectrum to track quench; it is most accurate with high-count rate samples. The range of SIS values reflects the energy range of the isotope. Both can be used as QIP's to create quench curves, although use of the external standard is preferred for samples containing low activity and is required for multilabeled samples.

Quench curve

A quench standard curve is a series of standards in which the absolute radioactivity (DPM) per vial is constant and the amount of quench increases from vial to vial. A quench curve uses the relationship between counting efficiency and QIP to correct the measured CPM to DPM. When a quench curve is made, the DPM value in each standard is known. Each standard is counted and the CPM is measured. The counting efficiency is calculated using the following relationship:

$$\frac{\text{CPM} \times 100}{\text{DPM}} = \% \text{ Counting Efficiency}$$

At the same time, the QIP is measured for each standard. A correlation is made using the QIP on one axis (X) and the % efficiency on the other axis (Y). A curve is fitted to the standard points. Figures 2 and 3 show quench curves for ³H and ¹⁴C using SIS as the QIP and quench curves for the same isotopes using t-SIE as the QIP. Once the quench curve is stored in the instrument computer, it can be used for automatic DPM calculations.

When unknowns are counted, the sample CPM and the QIP are measured. Using the QIP, the counting efficiency is determined from the quench curve. Sample DPM are then calculated by applying the appropriate efficiency to the CPM of the sample.

$$\text{DPM} = \frac{\text{CPM}}{\text{Efficiency (expressed as a decimal)}}$$

The standards and unknowns must be counted with the same energy regions. Revvity's LSC's with spectra based libraries (2500 series, 2700 series and the new 2900 and

3100 series) store the curve in a $0-E_{max}$ window and allow the curve to be recalculated for the windows used in the protocol. For other LSC's (1600,1900, 2100, 2200 and 2300) the windows used to acquire the quench curve must be used in the actual DPM determination.

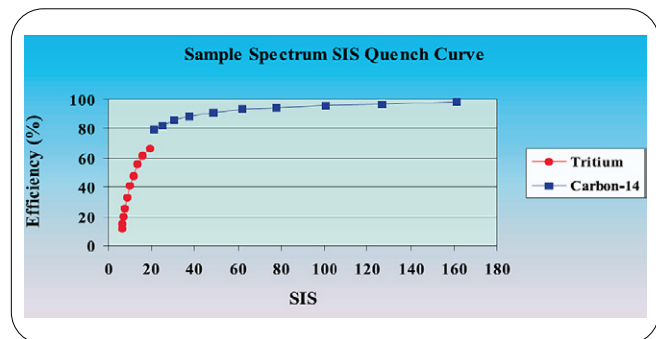


Figure 2. Quench Curves for ^3H and ^{14}C Using SIS as the QIP

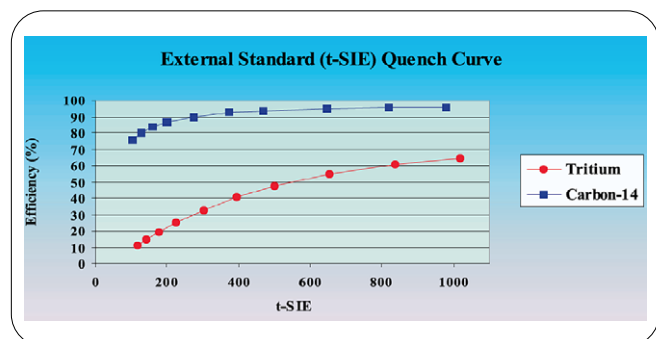


Figure 3. Quench Curves for ^3H and ^{14}C Using t-SIE as the QIP

Preparation of quench curves

Method 1

As mentioned, a quench standard curve is a series of standards in which the absolute radioactivity (DPM) per vial is constant and the amount of quench increases from vial to vial. The quench is increased from vial to vial by the addition of a quenching agent. A quenching agent is any chemical or color material added to the vial which causes a shift in the standard spectrum to a lower energy and a subsequent decrease in the counting efficiency of the radioactive standard. Usually a series of six to ten quench standards are prepared per radionuclide. This series is sometimes referred to as the quench set.

Revvity, Inc. provides factory stored quench curves in the instrument for ^3H and ^{14}C . On occasion, it is necessary for the investigator to prepare a quench curve, for isotopes other than ^3H or ^{14}C , (i.e., ^{35}S). There are some basic considerations to note before preparing a quench curve:

It is necessary to obtain a calibrated source of radioactivity to use as the source of the activity (DPM). It is essential that a known amount of activity be added per vial. Also, the standard material must be compatible with the cocktail chosen.

A suitable quenching agent must be chosen. It is desirable to closely approximate the chemical environment in the samples. If samples contain water with various other constituents, add the same material in increasing amounts to the standards. Additional quenching agents that are most often used and available in the laboratory are carbon tetrachloride (CCl_4), acetone ($\text{CH}_3\text{CH}_3\text{CO}$), chloroform (CHCl_3) and nitromethane (CH_3NO_2). To prepare the quench standards, perform the following steps:

1. Make a batch of radioactive solution in the chosen cocktail so the desired DPM are transferred to each individual vial when dispensing the cocktail. Prepare the standards with a sufficient level of activity, typically 50,000 to 200,000 DPM per vial, in order to be able to count the standards with good statistics in a short time. If ten standards are to be made with 10 mL of cocktail per vial, then 100+ mL of radioactive cocktail solution are required. If 15 mL of cocktail is to be used then 150+ mL of radioactive cocktail is required. Note: If the unknowns to be counted contain two radioisotopes (i.e., ^3H and ^{14}C), then individual standard curves must be prepared for each isotope.
2. Count the individual standards for at least five minutes to check for constant activity (CPM). Any sample that deviates more than 2% from the mean should be discarded.
3. Add incremental amounts of the quenching agent to vials 2 ... n (*quenching agent is not added to vial 1*) to obtain the desired quench range. It may be necessary to predetermine the amounts to add per vial by testing various volumes of quenching agent added to the cocktail only (no radioactivity) and monitor the amount of cocktail quench using t-SIE. Otherwise, add the suggested amounts of nitromethane based on the information given in Table 1.
4. Count the complete set under the conditions described in the instrument operation manual for storing a quench curve. Practically we suggest that the standards are counted to a preselected level of statistical accuracy (generally $0.5\%2s$), and this is usually achieved within five minutes per sample with the sealed standards which we provide.
5. Once the quench curve(s) are counted and stored, count unknown samples using the stored quench curve(s) to determine the DPM value for each sample.

Table 1. Volume of Nitromethane Needed for Quench Curve

Quench level	Toluene standards (15 mL)	Ultima Gold standards (15 mL)
A (1)	0 µL	0 µL
B (2)	1 µL	5 µL
C (3)	5 µL	10 µL
D (4)	11 µL	15 µL
E (5)	17 µL	26 µL
F (6)	25 µL	45 µL
G (7)	35 µL	70 µL
H (8)	45 µL	110 µL
I (9)	55 µL	150 µL
J (10)	66 µL	230 µL

Method 2

The preparation of a quench curve with any LSC cocktail is relatively straightforward and the following procedure is given as a guideline. Many researchers use their own methods and equipment and the procedure is, therefore, open to modification.

1. Dispense 10.0 mL or 15.0 mL of LSC cocktail into ten high performance glass vials.
2. Add activity to each vial (200,000 DPM for 3H or 100,000 DPM for ¹⁴C).
3. Count all ten vials to ensure that the same amount of activity is in each vial. A count time of about five minutes per vial will be sufficient. Any sample that deviates more than 2% from the mean should be discarded.
4. Number the vials 1 to 10 or A to J and add the suggested amounts of nitromethane based on the information given in Table 1.
5. Count the complete set under the conditions described in the instrument operation manual for storing a quench curve. Revvity recommends counting each standard for 30 minutes or until a pre-selected level of statistical accuracy (using %2S terminator, generally 0.5%) is reached.

Notes

1. For dispensing the activity, use a glass barreled microliter syringe fitted with a Chaney adapter. Such an adapter ensures reproducible dispensing of activity.
2. After preparation the standards should be stored in the dark preferably at 5°C to 10°C for best stability.

Notes on using the quench curves

1. t-SIE is independent of the sample isotope and of the activity in the vial, and has a large dynamic range. This makes it a very reproducible means of tracking quench in the cocktail.
2. SIS should only be used when there is at least 500 CPM activity in the sample. Remember that SIS uses the sample isotope spectrum to track quench; it is most accurate with high-count rate samples. For an accurate SIS, a good sample spectrum needs to be acquired.
3. SIS should not be used for low activity samples since an accurate sample spectrum cannot be acquired.
4. Most customers prefer to purchase quench standards. For cocktails based on toluene, xylene, pseudocumene or LAB (linear alkyl benzene) as the solvent, toluene quench standards should be used. For cocktails based on DIN (di-isopropyl naphthalene) or PXE (phenylxylethane) as the solvent, Ultima-Gold™ quench standards should be used. If the wrong quench standard is used there can be an error in DPM. This error is most pronounced with low energy isotopes such as tritium (see Tables 2-5).
5. Be sure that your prepared quench curve covers a wide t-SIE range (i.e., 800-300) in order to provide accurate DPM results.

Color quench

When a small amount of color is present in a sample there is virtually no difference between chemical and color quenching and the standard chemical quench curves are suitable. This applies to samples where the t-SIE is in the range 100 to 400. However, if a significant amount of color is present in the sample (t-SIE is <100) it may be necessary to consider preparing a color quench curve. When preparing a color quench curve, the selection of a suitable color quench agent is important. Aqueous soluble food dyes are usually a good choice as they provide both a stable color and a wide range of colors. The color of the sample must match the color used in the quench curve. Do not use a pH indicator since some cocktails contain acidic components and these will alter the color. Preparing a color quench curve is very similar to the method used for a chemical quench curve and the only difference is the quench agent. If considerable color is present in the sample it may be wise to modify or change the sample preparation method to either remove or reduce the level of color. Some techniques that have proved useful are shown in Table 2. The easiest way to reduce color quench is to either decrease the sample size or increase the cocktail volume or both.

Table 2. Techniques for Reducing Color in Certain Samples

Nature of sample	Suggested Remedy
Color from sample solubilization	Treat with hydrogen peroxide to bleach out the color
Plant material	Consider sample oxidation
Inorganic matrix	Change to alternate colorless anion

Quench curve errors

The errors that can be present when the wrong quench curve is used with an LSC cocktail are shown in the following tables:

Table 3. Various Cocktails vs. Ultima Gold Quench Curve (Tritium)

Tritium:	Ultima Gold	Toluene	Opti-Fluor	Insta-Gel Plus	Pico-Fluor 15
No quench	-0.12%	-1.04%	6.00%	2.70%	4.89%
Low quench	-0.46%	4.24%	7.06%	5.14%	6.45%
Medium quench	0.04%	5.87%	8.43%	5.82%	6.91%
High quench	-0.14%	10.10%	14.41%	10.02%	11.89%
Highest quench	-0.20%	13.42%	18.01%	13.36%	13.43%

Table 3 shows that only Ultima Gold cocktails should be used for Tritium DPM measurements with the Ultima Gold Tritium quench curve.

Table 4. Various Cocktails vs. Toluene Quench Curve (Tritium)

Tritium:	Ultima Gold	Toluene	Opti-Fluor	Insta-Gel Plus	Pico-Fluor 15
No quench	-4.10%	-0.49%	-0.57%	-1.58%	-0.32%
Low quench	-5.33%	-0.27%	-0.13%	-1.22%	0.23%
Medium quench	-6.51%	0.01%	0.45%	-0.19%	0.60%
High quench	10.39%	-0.01%	1.21%	-0.79%	-0.49%
Highest quench	-16.16%	-0.70%	0.11%	0.56%	-0.21%

Table 4 shows that the Ultima Gold cocktails should not be used for Tritium DPM measurements with the Toluene Tritium quench curve.

Table 5. Various Cocktails vs. Ultima Gold Quench Curve (Carbon-14)

Carbon-14:	Ultima Gold	Toluene	Opti-Fluor	Insta-Gel Plus	Pico-Fluor 15
No quench	0.06%	-1.37%	2.51%	2.25%	1.96%
Low quench	0.03%	0.12%	2.04%	0.78%	0.80%
Medium quench	-0.13%	0.84%	1.72%	1.15%	1.20%
High quench	0.02%	1.30%	2.51%	3.11%	1.71%
Highest quench	-0.63%	4.52%	3.81%	3.59%	2.77%

Table 5 shows that only Ultima Gold cocktails should be used for Carbon-14 DPM measurements with the Ultima Gold Carbon-14 quench curve.

Table 6. Various Cocktails vs. Toluene Quench Curve (Carbon-14)

Carbon-14:	Ultima Gold	Toluene	Opti-Fluor	Insta-Gel Plus	Pico-Fluor 15
No quench	0.45%	0.42%	1.71%	1.90%	1.96%
Low quench	0.27%	-0.03%	0.89%	1.20%	0.43%
Medium quench	-0.54%	-0.37%	1.28%	0.31%	0.83%
High quench	-0.81%	0.01%	1.86%	0.77%	0.81%
Highest quench	-16.51%	0.33%	1.04%	0.49%	0.88%

Table 6 shows that the Ultima Gold cocktails should not be used for Carbon-14 DPM measurements with the Toluene Carbon-14 quench curve.

Using a quench curve

Once the quench curve is stored in the instrument computer, it can be used for automatic DPM calculations. When unknowns are counted, the sample CPM and the QIP are measured. Using the QIP (SIS or t-SIE) the counting efficiency is determined from the appropriate quench curve.

For example:

A Tritium sample is analyzed and has:

$$\text{Count rate} = 10,000 \text{ CPM}$$

$$t\text{-SIE} = 400$$

From the quench curve shown in Figure 4. The instrument uses the t-SIE of 400 to determine that the counting efficiency is 42%. Since we now know the CPM and the counting efficiency it is possible to calculate the DPM:

$$\begin{aligned} \text{DPM} &= \frac{\text{CPM}}{\text{Efficiency (expressed as a decimal)}} \\ &= \frac{10,000}{0.42} \\ &= 23,809 \text{ DPM} \end{aligned}$$

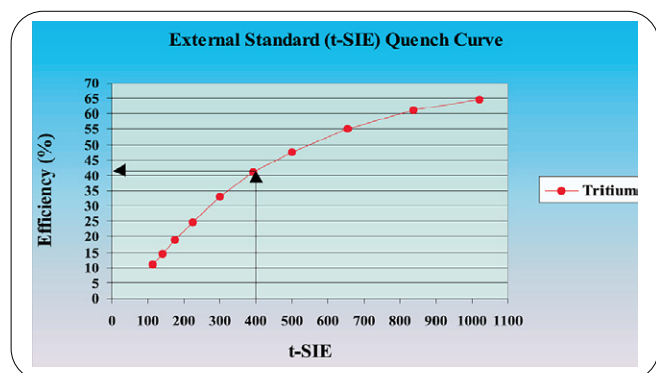


Figure 4. How the Instrument Uses t-SIE to Determine Efficiency

Summary

A compilation of recommended quench curves for different LSC cocktails are shown in Table 7. This will provide a basic guide to correct quench curve selection.

Table 7. A Simple Guide to Quench Curve Selection

Cocktail	Recommended Quench Curve (³ H AND ¹⁴ C)
Ultima Gold™	Ultima Gold
Ultima Gold AB	Ultima Gold
Ultima Gold LLT	Ultima Gold
Ultima Gold MV	Ultima Gold
Ultima Gold XR	Ultima Gold
Ultima Gold F	Ultima Gold
StarScint	Ultima Gold
Opti-Fluor®/Opti-Fluor O	Toluene
Emulsifier-Safe™	Toluene
Insta-Gel® Plus	Toluene
Pico-Fluor™ 15	Toluene
Pico-Fluor 40	Toluene
Insta-Fluor™	Toluene
Hionic-Fluor™	Toluene
Filter-Count™	Toluene
Carbo-Sorb® E / Permafluor® E+	Toluene
Monophase® S	Toluene
Formula 989	Toluene
AQUASOL™/AQUASOL II	Toluene
AQUASSURE®/BIOFLUOR®	Toluene
ATOMLIGHT®	Toluene
ECONOFLUOR®-2	Toluene

Conclusion

For accurate DPM recovery, it is imperative that the quench curve selected is appropriate for the LSC cocktail being used. Revvity manufactures quench curves using either a safer, high flash point (Ultima Gold) cocktail or a classical solvent (Toluene) that are suitable for use with all Revvity's LSC cocktails. If problems with quench curve selection or preparation persist, or help is needed with a particular isotope not mentioned in this publication, please call your local Revvity representative for further applications support.

References

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3. Ross, H.H. 1965. Color quench correction in liquid scintillator systems. *Analytical Chemistry*, 37(4): 621-623.
4. Ten Haaf, F.E.L. 1975. Color quenching in liquid scintillation counters. In Crook, M.A. and Johnson, P., eds., *Liquid Scintillation Counting*, Vol. 3. London, Heyden & Son, Ltd.: 41-43.

The application note information enclosed is used to illustrate the technique and may not represent the latest instrument, reagents and cocktails. Customers should validate the technique in their laboratory.

Contact Customer Care at www.revvity.com

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