APPLICATION NOTE

Requirements for a “good“ measurement with a liquid scintillation counter.

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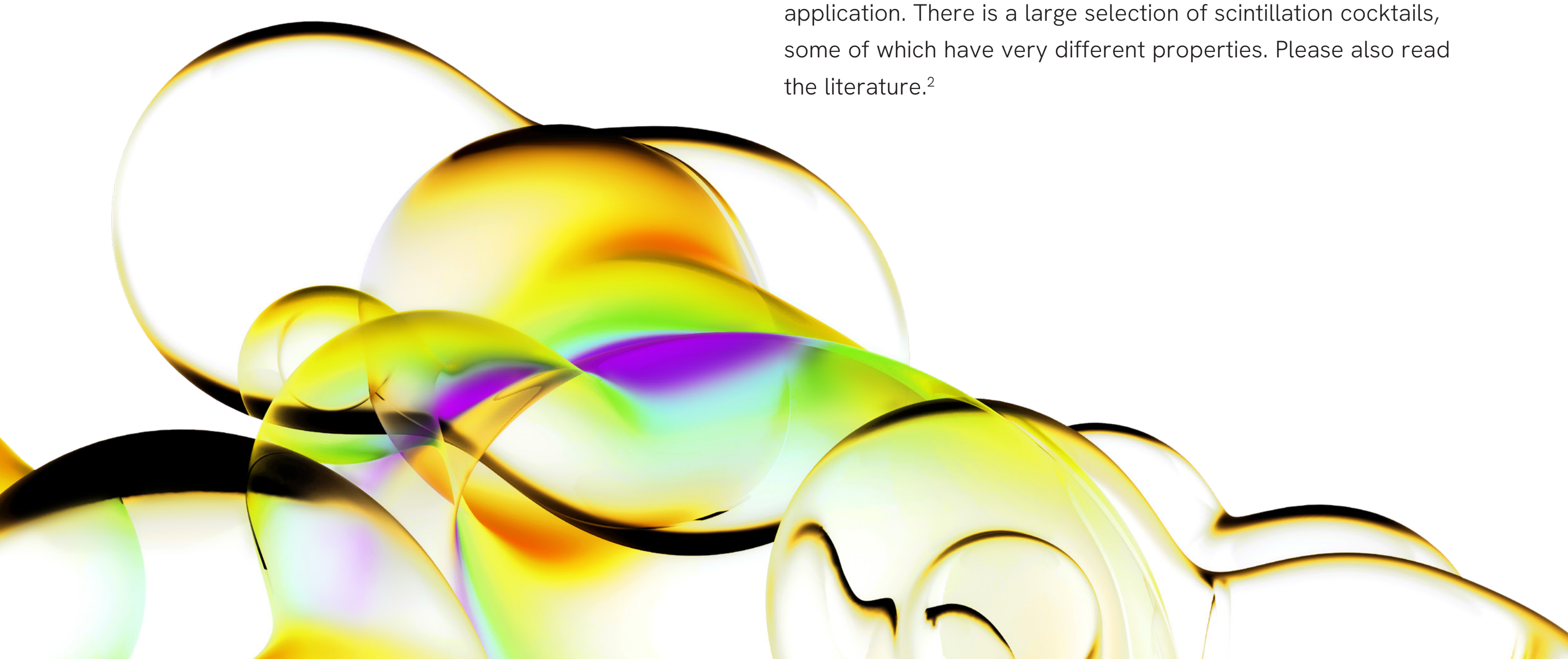
Introduction

First, it must be clarified what we mean by a good measurement. This question is not easy to answer. In the context of this application note, we want to mean a correct result with the least possible uncertainty. The requirements for a correct result and the uncertainty can vary greatly depending on the user and the task at hand, but fundamentally certain things must be observed for sensible measurements. Of course, this includes sample preparation, maintenance of the scintillation counter, sensible settings in the measurement protocol, implementation of necessary corrections such as background correction, quench correction, etc., possibly also method optimization and, finally, sensible data output.

Sample preparation

In the simplest case, sample preparation can be dissolving the sample in a scintillation cocktail. However, sample preparation is often more complex and can include filtrations, distillations, extractions, decolorization and many other steps that can hardly be listed here. An overview of the sample preparation but also of the scintillation technique was recently published in a book.[1] The aim of all sample preparation steps should be to obtain a sample that is clear, as colorless as possible and free of solids or suspended particles. At least one should be sure that these particles dissolve in the cocktail. After adding the cocktail, the sample must result in a homogeneous solution to avoid self-absorption in the sample. Cloudy, milky samples or samples that form two phases must be avoided. Also ask the manufacturer of cocktails about the ideal cocktail for your application. There is a large selection of scintillation cocktails, some of which have very different properties. Please also read the literature.[2]



Especially with complex sample preparations, it is important to run a blank sample without activity but otherwise with the same composition through all sample preparation steps. Such a sample can later be used as a background sample. In some cases, samples with known activity (tracer) may also have to go through all steps in order to be able to record possible losses during sample preparation.

Maintenance of the scintillation counter

There is not much that the user can do, but there are some things that should be done regularly. First, the sample space should be checked for possible contamination, and this should be removed with a slightly damp cloth, if necessary.

In addition, the liquid scintillation counter should be normalized and calibrated regularly using the SNC protocol.

It is also necessary to check whether the expiry date for the standards has already been reached or even exceeded. Then new standards should be ordered immediately. These standards must be completely unquenched. They are in a protective gas atmosphere in the glass vials which are closed by Revvity by molten the glass, but their shelf life is still limited to around 5 years. After that, the quench increases in the samples, which can lead to incorrect evaluations. During calibration, among other things, a completely unquenched sample is assigned the tSIE value of 1000. If the quench of a standard that is too old increases, the tSIE value of 1000 is assigned to a quenched sample. This means that when quench curves are used, the sample is assigned a counting efficiency that is too high and activities that are too low are then determined.

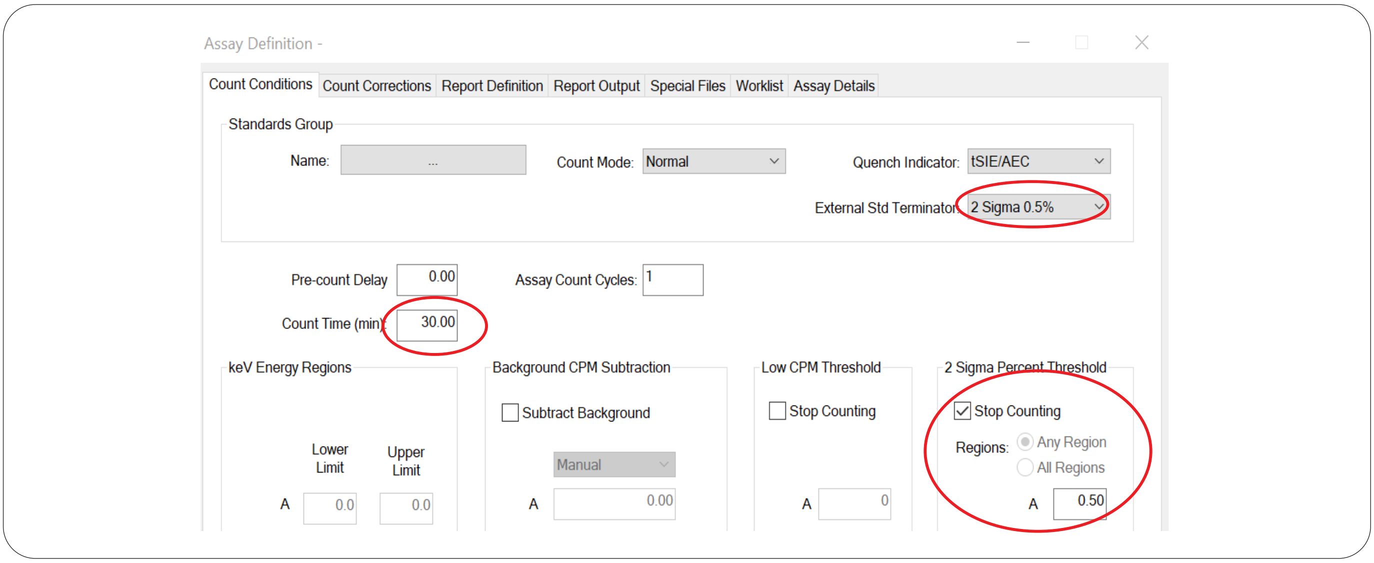
In addition to assigning the tSIE value, several other things are also performed during normalization and calibration, and correct execution is of crucial importance.

This normalization and calibration should be done about once a week with the factory installed SNC protocol carried out so that the device is always ready for use.

The quench curves should also be renewed regularly if quench curves are used. Changes over the years of the device and cannot correct all effects via the SNC protocol. It is therefore recommended to recreate the quench curves once a year. This can also be recommended after a visit by the service technician, especially if the last service call was a long time ago. In that case, when a cleaning of the measurement chamber and changing the reflectors is done the counting efficiency of the instrument can increase significantly. New quench curves must be recorded so that quench curves still lead to correct results in such a case.

Settings in the protocol to obtain a quench curve with good accuracy and precision

The practical creation of a quench curve is described in detail in literature.[3] This is now more about the settings that are required to obtain a quench curve with low uncertainty. Three settings on the side of the software shown in Figure 1 are important, two of which are already preset. On the one hand, the External Std Terminator (external ^{133}Ba source) is preset to 2σ 0.5%, and on the other hand, Stop Counting ends the measurement when the quenched standards reach an error of 0.5% in the 2σ confidence interval.



Name: ...

Count Mode: Normal

Quench Indicator: tSIE/AEC

External Std Terminator: 2 Sigma 0.5%

Pre-count Delay 0.00

Assay Count Cycles: 1

Count Time (min): 30.00

keV Energy Regions: Lower Limit A 0.0, Upper Limit A 0.0

☐ Subtract Background

Manual

A 0.00

☐ Stop Counting

A 0

☑ Stop Counting

Regions: ● Any Region ○ All Regions

A 0.50

Figure 1: Assuring good counting statistics for the measurement of standards

However, this setting does not mean that an error of 0.5% is also achieved for the sample, because there is a second termination criterion for the measurement, the measurement time. The default is 30 minutes. The measurement is ended when at least one criterion, the measurement time or the error is reached. The measurement can therefore be aborted when the error of 0.5% is reached, but at the latest when the specified measurement time is reached, in some circumstances with a larger error. If an error of 0.5% is to be achieved, as is recommended when recording quench curves, then a sufficiently long measurement time should be selected. With the commercial quench curves available from Revvity, this is usually possible with a measurement time of 40 minutes, even for ^{3}H quench curves. If you create a quench curve yourself, you have to set the measurement time so that you can achieve a total of 160,000 counts with the most heavily quenched standard. If your most heavily quenched sample has a count rate of 10,000 CPM, you would have to set a measurement time of at least 16 minutes in order to achieve 160,000 total counts. With this number of counts you achieve a statistical error of 0.5% in the 2σ confidence interval. The default counting time for the external ^{133}Ba-sourve should not be changed for Tri-Carb scintillation counters. This is different when using a Quantulus GCT. Here the counting time should better be set to 10 minutes. This is necessary because the activity of the ^{133}Ba source in the Quantulus is almost 20 times smaller than in the Tri-Carb. The contribution of the sample, which is always measured together with the ^{133}Ba source, is significant in the Quantulus when using quenched standards and cannot be practically neglected, as with the Tri-Carb. With the default setting of 0.5% error, which relates to the entire activity in the measuring chamber, a higher error would be achieved for the ^{133}Ba source in the Quantulus GCT alone.

Settings in the protocol for unknown sample measurements

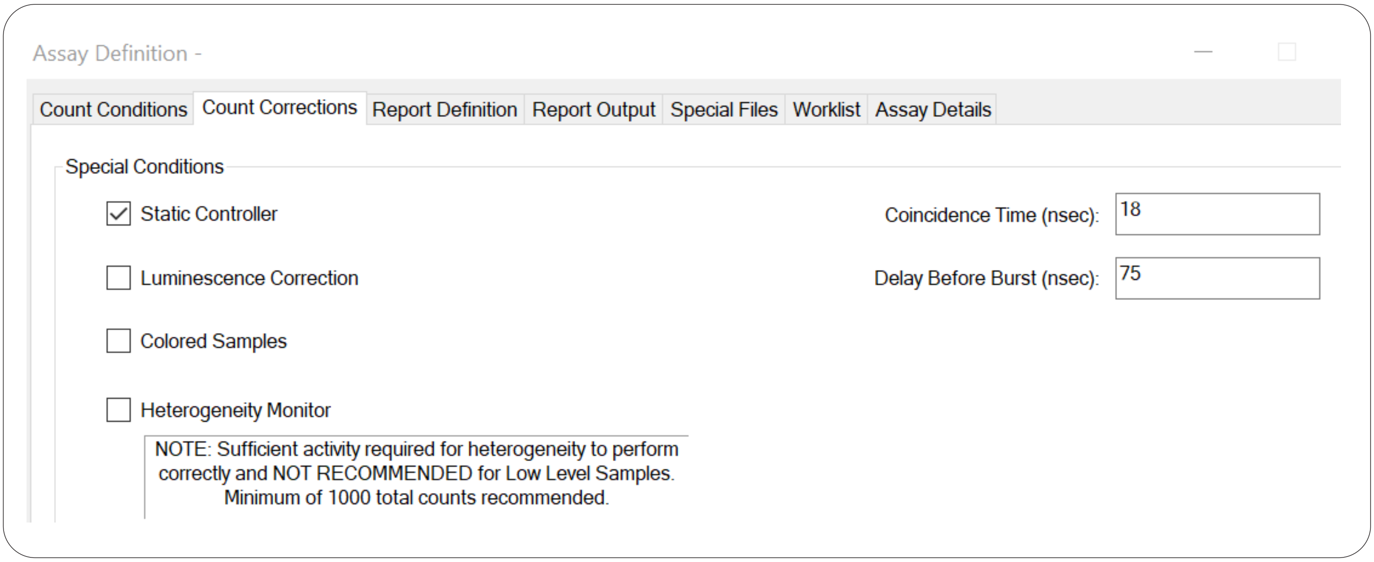
When measuring the unknown samples, the same considerations apply to the measurement of the external ^{133}Ba source as were already made for the measurement of the quench curve, if quench curves are to be used. In this case it is important that a measurement protocol is linked to a suitable quench curve. There are basically two types of quench curves. Some have been prepared with the older classic solvents such as toluene, xylene or pseudocumene (1,2,4-trimethylbenzene). These quench curves may only be used for samples whose cocktails are also based on these solvents. Newer, so-called safer cocktails are often based on di-isopropyl naphthalene or phenylxylylethane (PXE) and similar solvents and should only be used with quench curves based on cocktails from the Ultima Gold series or other safer cocktails. If an incorrect quench curve is linked to the protocol, considerable errors of up to 20% can occur, especially with low-energy nuclides such as ^{3}H, in heavily quenched samples. For the possible errors, see also examples in the literature[1,3].

Correction of background

Background correction is required, especially for samples with count rates only slightly above the background, so that the activity of the sample is not overestimated. The background deduction can be activated in the protocol in the Assay Definition window on the Count Conditions page of the QuantaSmart software. The "1st Vial" option is usually the only sensible one and uses the sample in position 1 of the sample rack as a background sample. Details of the preparation of a good background sample should not be given here, as this is described in detail in literature.[4] It should only be mentioned here that the background sample should show a tSIE quench parameter like the unknown samples.

Elimination of static charge

In addition to the already mentioned quench correction and a background correction, the elimination of static charges is very important in plastic scintillation vials. For this reason, the static controller should always be active as shown in Figure 2. The static controller ionizes the air in the elevator shaft and samples that get through this shaft into the measurement chamber are freed from external charge on the scintillation vial.



Special Conditions

☑ Static Controller

☐ Luminescence Correction

☐ Colored Samples

☐ Heterogeneity Monitor

NOTE: Sufficient activity required for heterogeneity to perform correctly and NOT RECOMMENDED for Low Level Samples. Minimum of 1000 total counts recommended.

Coincidence Time (nsec): 18

Delay Before Burst (nsec): 75

Figure 2: The Count Corrections tab of the protocol

Correction of luminescence

Luminescence increases the count rate and leads the user to believe that the sample activity is higher than what is present. Today's modern scintillation counters allow the user to correct almost everything and that fully automatically. However, every user should be clear about what these corrections look like and what consequences this has on his measurement result. I would like to mention our training classes in Hamburg again, in which we show the possibilities and limits of the scintillation counter. In fact, the luminescence correction works very well, but like any correction, this correction is also subject to an error, at least a statistical error, and thus the uncertainty of the result is increased. I am therefore not a fan of the luminescence correction, especially when it comes to very low activities in the samples. An alternative can be to leave the sample in the dark in the scintillation counter for a while before starting the actual measurement. This solution is particularly recommended for low-energy nuclides such as ^{3}H. Of course, when preparing the sample, care should be taken to ensure that no luminescence is provoked, for example by using hydrogen peroxide to bleach colored samples. In such a case, it is important to carry out the procedures carefully and to destroy excess peroxide after bleaching. Details on this can be found in literature.[5]

Another possibility for eliminating luminescence is to exclude these photons through the choice of the energy window. Luminescence is a very low-energy event that occurs primarily in the 0-5 KeV range. If higher-energy nuclides such as ^{14}C, ^{35}S, ^{33}P etc. are measured, the range from 0-5 KeV can often be dispensed with without reducing the sensitivity. In fact, this increases the sensitivity, because the background is also particularly high in this energy range of 0-5 KeV and the signal-to-noise ratio can often be improved by excluding this range.

However, this type of correction is not suitable for the low energy ^{3}H nuclide, since the counting yield would decrease too much if the range from 0-5 KeV were omitted.

Optimization of energy windows

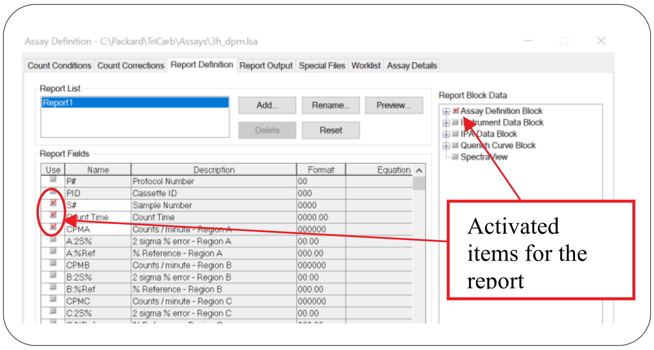
In which energy window should a sample be measured? I noticed that many customers measure the nuclides very often in the routine in the open energy window, e.g. B. ^{3}H from 0-18.6 KeV, ^{14}C from 0-156 KeV etc. However, since almost all samples are subject to a certain quench, the energy distributions often end before the maximum value of the open window and in this area there is often only background but no sample signal measured. In addition, the background is highest in the very low-energy range and the signal-to-noise ratio can often be improved by excluding some KeV from the evaluation at the beginning of the energy distribution. This results in a lower counting efficiency with a simultaneous reduction in the background. When optimizing the energy window, it is important to set the energy window in such a way that the background decreases significantly without reducing the counting efficiency too much. Such optimization tasks can be taken over by simple programs in a fraction of a second.

Only a background spectrum and a sample spectrum are required for this, but both should show approximately the same quench, i.e. comparable tSIE values. Currently, all Quantulus GCT and Tri-Carb systems are shipped with the SpectraWorks2 software. With this software, the specified spectra can be loaded and the optimal energy window for the measurement can be determined in just a few steps. Details on the procedure can be found in literature.[6,7] The so-called Figure of Merit (FOM) is optimized in the SpectraWorks2 software. It results from the counting efficiency in percent squared divided by the background in CPM (E^2/B). Even without SpectraWorks2 software, this value can of course be determined manually by measuring both the background sample and the measurement sample with the same quench in the samples in different measurement windows and the figure of merit is determined for each measurement window according to the above equation.

The maximum value for the FOM indicates the optimal measurement window. More detailed information about the optimization of measurement protocols can be found in the literature.[1]

Report creation

The report is created in the protocol on the "Report Definition" tab. Here, data from the "Report Fields" table as well as from the "Report Block Data" area can be activated for the report.



Assay Definition - C:\Packard\TriCarb\Assays\3h_dpm.lsa

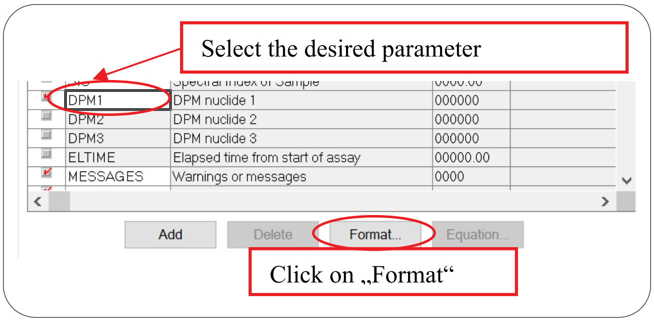
Use	Name	Description	Format	Equation
☐	P#	Protocol Number	00	
☐	PID	Cassette ID	000	
☑	S#	Sample Number	0000	
☑	Count Time	Count Time	0000.00	
☑	CPMA	Counts / minute - Region A	000000	
☐	A:2S%	2 sigma % error - Region A	00.00	
☐	A:%Ref	% Reference - Region A	000.00	
☐	CPMB	Counts / minute - Region B	000000	
☐	B:2S%	2 sigma % error - Region B	00.00	
☐	B:%Ref	% Reference - Region B	000.00	
☐	CPMC	Counts / minute - Region C	000000	
☐	C:2S%	2 sigma % error - Region C	00.00	

Report Block Data
- ☑ Assay Definition Block
- ☐ Instrument Data Block
- ☐ IPA Data Block
- ☐ Quench Curve Block
- ☐ SpectraView

Activated items for the report

Figure 3: The "Report Definition" tab

In addition to the choice of the important parameters for the report such as CPM, DPM, Bq or the quench parameter, the accuracy is also often important. When it comes to measurements close to the background, it can be important to consider one or two decimal places. By default, no decimal places are output. For example, if you want to output two decimal places for the DPM1 values, proceed as shown in Figure 4.



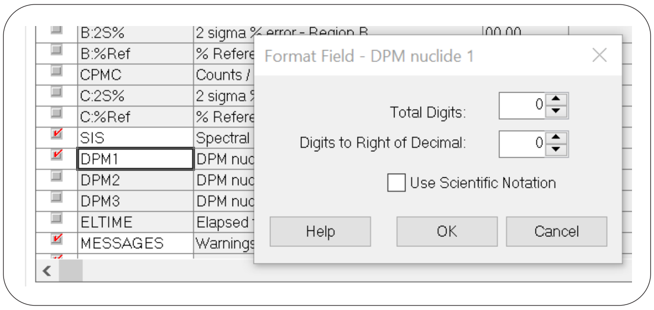
Select the desired parameter

☑	DPM1	DPM nuclide 1	000000
☐	DPM2	DPM nuclide 2	000000
☐	DPM3	DPM nuclide 3	000000
☐	ELTIME	Elapsed time from start of assay	00000.00
☑	MESSAGES	Warnings or messages	0000

Click on „Format"

Figure 4: "Report Definition" tab

As soon as you click on the "Format" field, the following box appears in which you can enter the number of total characters and the number of digits after the decimal point.



Format Field - DPM nuclide 1

Total Digits: 0

Digits to Right of Decimal: 0

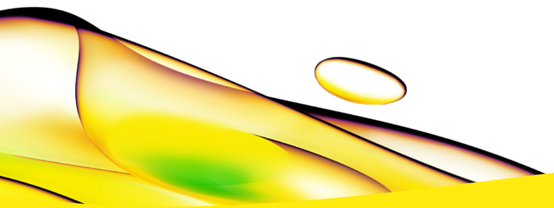
☐ Use Scientific Notation

Figure 5: Entry of decimal places

In addition, the report can of course contain a lot more information. Different reports can be created for a measurement, formulas can be integrated, and the reports can be saved in different formats.

Literature

1. R. H. W. Edler; An Introduction to the Scintillation Technology for the Measurement of Radionuclides, 1st Edition, Bremen 2020, ISBN 978-3-00-020422-7.
2. R. Edler; "Cocktails for Liquid Scintillation Counting", Revvity LAS (Germany) GmbH, January 2018.
3. J. Thomson; "Use and Preparation of Quench Curves in Liquid Scintillation Counting", Revvity LAS (Germany) GmbH, January 2018.
4. R. Edler; "Background Correction and Dependency on Quench", Revvity LAS (Germany) GmbH, October 2021.
5. J. Thomson, D. A. Burns "Solubilization as Sample Preparation for Liquid Scintillation Counting", Revvity LAS (Germany) GmbH, August 2017.
6. R. Edler; "The New SpectraWorks2 Software", Revvity LAS (Germany) GmbH, July 2017.
7. R. Edler; "Protocol Optimization for ^{3}H, ^{14}C and ^{3}H/^{14}C Dual Label Applications using a Quantulus GCT", Revvity LAS (Germany) GmbH, October 2020.



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