TopCount

TCA-028

Dual Label DPM on the TopCount™

Introduction

In dual label assays, the two isotopes are detected at different efficiencies due to the different energies of their beta emissions. Because the energy of a beta particle is distributed from 0 to E_{max}, overlap of the two isotope spectra always occurs. DPM correction algorithms subtract the contribution caused by this overlap (spill) and adjust for the relative efficiencies. The use of DPM allows for meaningful interpretation and comparison of results in a dual label assay.

There are two methods for performing dual label DPM on the TopCount. The first is for samples that have variable levels of quench. This method requires the use of an external standard and the creation of two single label quench standard sets for DPM correction. The external standard option is available with the 24-well plate format in the TopCount. The second method of dual label counting assumes that all the samples are prepared in the same manner, and have a constant level of quench. Efficiency values from known standards are used to correct CPM to DPM. This constant quench method can be used for samples in either the 24- or the 96-well format. This paper will discuss the constant quench method in detail. Variable quench dual label counting performance is presented in TopCount Topics TCA-015, "Quench and Quench Correction."

Constant Quench Dual Label DPM

The first step in setting up constant quench dual label counting is to determine the two appropriate counting regions. The lower energy isotope should be fully detected in the lower energy region. The region for the higher energy isotope should be set to exclude any counts from the lower energy isotope; that is, to exclude any spill up (see Figure 1). RegionView is a useful feature on the TopCount for determining the appropriate region settings. (Refer to the TopCount Reference Manual.) Once the settings are known, a dual label nuclide should be created in the Nuclide Library.

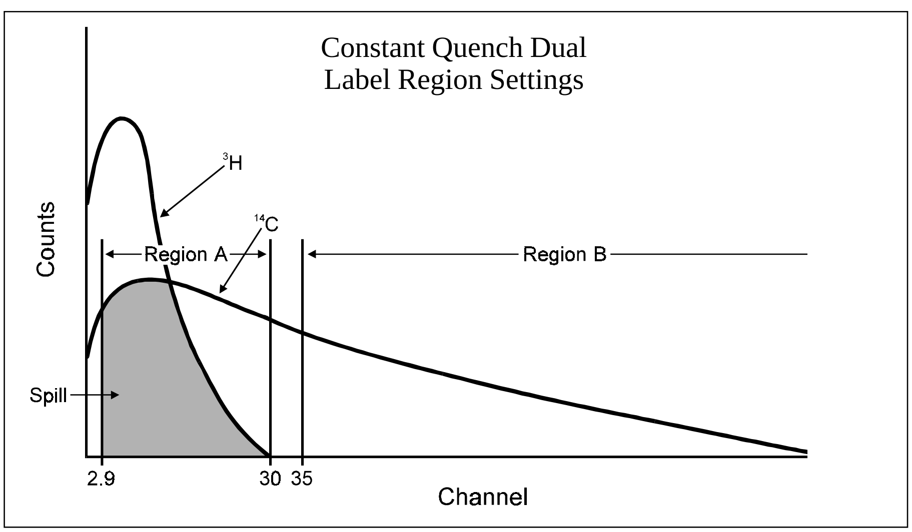

Figure 1.

Packard Instrument Company 800 Research Parkway, Meriden, CT 06450 Tel: 203-238-2351 1-800-323-1891
FAX: 203-639-2172 Web Site: http://www.packardinst.com Email: webmaster@packardinst.com

Packard Instrument, Co. SYSTEM CERTIFIED ISO 9001

PAN0090 12/96 Printed in U.S.A.

Example: For $^{3}H/^{14}C$ using MicroScint™ cocktail, version 4.0 or higher TopCount software.

Create a Custom Nuclide Setting

(See Figure 2)
From the STATUS window, press F1 - Directory, then highlight protocol of interest and press F3 - Count Conditions

Highlight the Radionuclide? line, and press F3 - Library

Select the function key F3 - Add Nuc to add a new nuclide setting

Type '$^{3}H/^{14}C$ Liquid' <enter>

Select 'Liq/Plast' scintillator, 'Low' energy, and 'Normal' counting mode

Region A: LL: type '2.9' <enter>, UL: '30' <enter>
Region B: LL: type '35' <enter>, UL: '256' <enter>

Press F1 to Exit/Save new settings

After defining the counting regions, individual standards of each isotope must be counted under these regions to determine the efficiencies, and spill factors for use in correcting CPM to DPM. The standards should be of known DPM and prepared under the same final chemical and physical conditions (same quench level) as the assay unknowns. After counting the standards, calculate the efficiency for the low energy isotope in Region A, the efficiency of the high energy isotope in Region B, and the spill. Spill is defined as the amount of high energy isotope that is measured in Region A.

The equations for these calculations are as follows:

1. ^{3}H Efficiency $= \dfrac{CPM_A}{DPM_{3H}} = EFF_{LA}$

2. Spill Factor $= \dfrac{CPM_A}{DPM_{14C}} = EFF_{HA}$

3. ^{14}C Efficiency $= \dfrac{CPM_B}{DPM_{14C}} = EFF_{HB}$

Note: LA = Low energy isotope in Region A
HB = High energy isotope in Region B
EFF_{HA} = Spill factor for high energy isotope into Region A

Using the values from the above equations, custom cells in the TopCount can be created to automatically provide the corrected DPM values on the disk file and printout. The equations for DPM (broken down into steps) are as follows:

1. $DPM_{14C} = CPM_B / EFF_{HB}$
2. $SPILL = DPM_{14C} * EFF_{HA}$
3. $CCPM^{\dagger}_{3H} = CPM_A - SPILL$
4. $DPM_{3H} = CCPM_{3H} / EFF_{LA}$

† CCPM = Corrected CPM

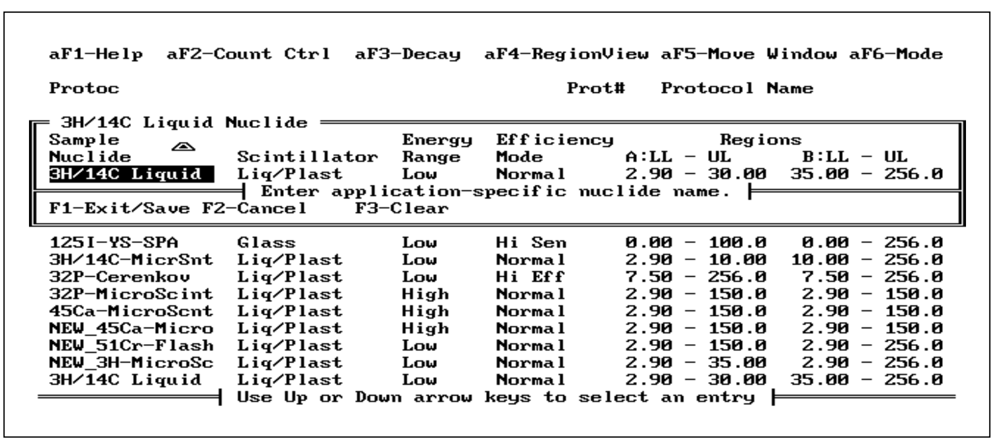

Figure 2.

These equations are then entered into the custom cells for the protocol of interest.

Custom Cell Definition

(See Figure 3)
From the Directory page, highlight the protocol of interest and press F8 - Format Data Cells. This opens the data cell edit window. (Use arrow keys to move the cursor in this window.)

- Scroll down past the preset cells and highlight the first custom cell (C# 20)
  Type “BXXXXXX” <enter> for format
  Highlight the name ‘Custom 1’ and type “DPM-14C” <enter>
- Type “C7/EFF_{HB}” <enter>; where EFF_{HB} is the decimal value determined above
- Move the cursor to the next custom cell
  Type “BXXXXXX” <enter> for format
  Highlight the name ‘Custom 2’ and type “SPILL” <enter>
  Type “C20∗EFF_{HA}” <enter>; where EFF_{HA} is the decimal value determined above
- Move the cursor to the next custom cell
  Type “BXXXXXX” <enter> for format
  Highlight the name ‘Custom 3’ and type “C-CPMA” <enter>
  Type “C6-C21” <enter>
- Move the cursor to the next custom cell
  Type “BXXXXXX” <enter> for format
  Highlight the name ‘Custom 4’ and type “DPM-3H” <enter>
  Type “C22/EFF_{LA}” <enter>; where EFF_{LA} is the decimal value determined above
  Press F1 - Exit to save changes and return to the Directory page

The cells containing the final result for DPM_{3H} and DPM_{14C} must be selected in the ‘Printer Output’ window (Figure 4) and the ‘Disk File Output’ window (Figure 5) to get the calculated values in the file or on the hard copy respectively.

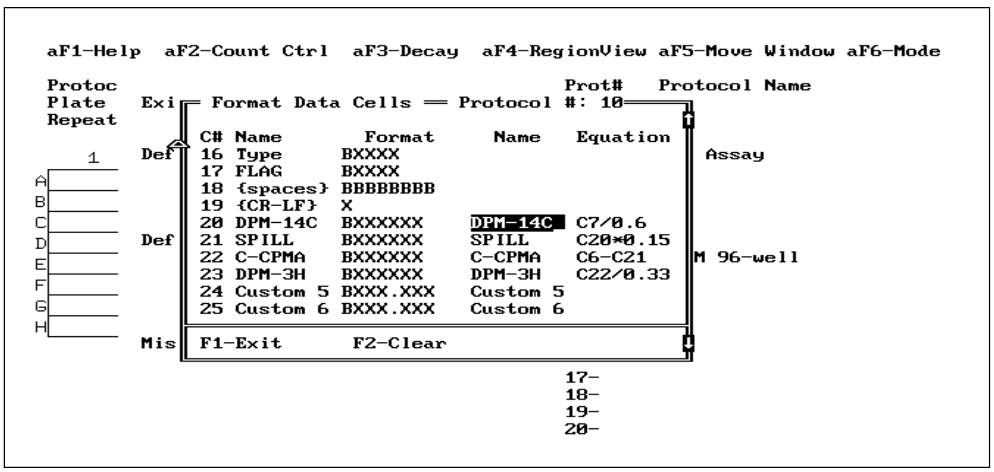

Figure 3.

Example: Constant Quench Dual Label DPM

The single label standards are counted using the nuclide setting described previously. Each standard is known to contain 100,000 DPM.

For the ^{3}H standard:
CPM_A = 33,000 and CPM_B = Background

For the ^{14}C standard:
CPM_A = 15,000 and CPM_B = 60,000

The efficiency and spill values are then calculated:

E_{HB} = 60,000/100,000 = 0.6 (60% efficiency)

E_{LA} = 33,000/100,000 = 0.33 (33% efficiency)

E_{HA} = 15,000/100,000 = 0.15 (15% efficiency)

An unknown sample containing ^{3}H and ^{14}C labeled material is counted and the results are as follows:

CPM_A = 10,000 CPM_B = 10,000

Are there equal amounts of both ^{3}H and ^{14}C in the sample?

DPM_{14C} = 10,000/0.6 = 16,667 DPM of ^{14}C

SPILL = 16,667 $*$ 0.15 = 2,500

$CCPM_{3H}$ = 10,000 - 2,500 = 7,500 CPM of ^{3}H

DPM_{3H} = 7,500/0.33 = 22,727 DPM of ^{3}H

Conclusion

In reality, there is almost 1.4 times as much ^{3}H present as ^{14}C in this sample. Using DPM allows for an accurate comparison of the amounts of the two isotopes in a given sample, and for comparison of values from different experiments. The custom cell feature on the TopCount provides a convenient, automatic means of performing constant quench dual label assays with high throughput for screening applications.

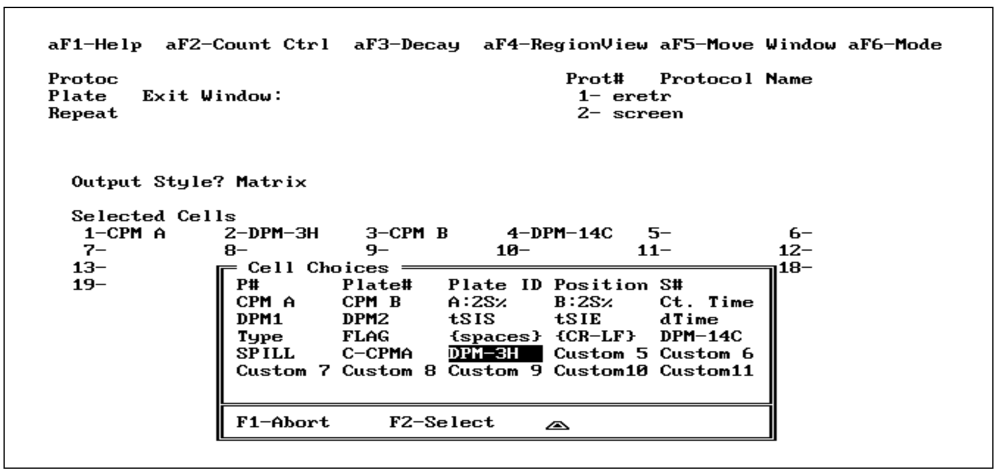

Figure 4.

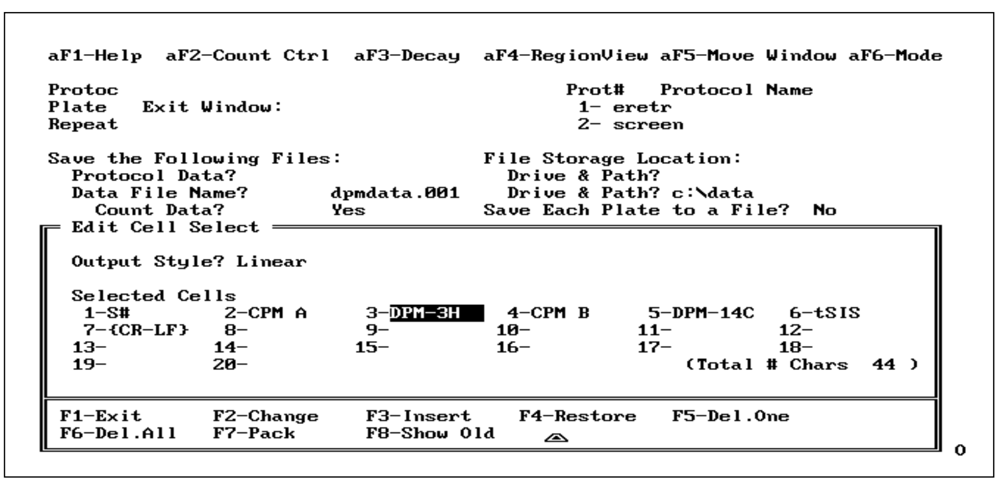

Figure 5.