APPLICATION OF NEW COUNTING MODES TO CYTOSTAR-T# CELL BASED ASSAYS

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Introduction

Cytostar-T™ scintillating microplates are standard format, sterile, tissue culture treated microplates designed for real-time analysis of a broad spectrum of cell associated phenomena. Radioisotopes brought into close proximity with the scintillant containing base, by virtue of the biological processes within cells, will generate light that can be quantified using appropriate instrumentation.

Both the Packard TopCount™ and the Wallac MicroBeta™ instruments can be used with Cytostar-T plates. With both instruments, new approaches have been developed to increase count rates in scintillation-type assays[1,2,3]. These are High Efficiency Count Mode (HECM) for the TopCount and ParaLux counting for the MicroBeta.

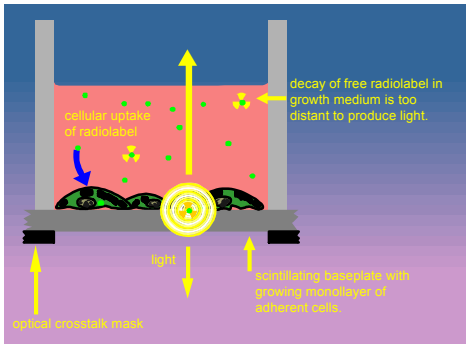

Figure 1. Cytostar-T scintillating microplate. The transparent base plate of each well is composed of a compounded scintillant and plastic mixture, compatible with the cultivation and observation of adherent cell monolayers. Radioisotopes having suitable decay characteristics can be brought into proximity with the scintillant containing base by virtue of the biological processes within the cells, thereby generating light. In contrast the decay of the free radiolabel in solution is too distant to produce a significant signal.

In the TopCount Normal Count Mode (NCM), detected events must display a two or three pulse signature to be accepted as a true decay. The instrument allows the discrimination of true events from background noise by analysing the differences in afterpulse characteristics of the scintillator as compared to one pulse background, which is composed mostly of photomultiplier tube (PMT) noise. This is the recommended mode for Cytostar-T assays when the signal is not too low.

However, for assays with low signals, HECM can be used to enhance the signal obtained. The instrument then uses one-pulse criteria to accept an event as being of true beta origin. Backgrounds are controlled by adjusting the lower level energy discriminator.

Background counts are typically of very low energy. In contrast, true counts appear as higher energy signals in the multichannel analyzer.

The MicroBeta TriLux instrument uses coincidence circuitry to distinguish a true signal from background noise. An event must be registered by two PMTs, one positioned above the sample and one below, within a specified time frame, to be registered.

ParaLux count mode simultaneously constructs two spectra of each sample. The first spectrum includes all the beta decay that is of sufficient energy to produce photons that are accepted by both PMTs (coincidence counts). The second spectrum includes the beta decay that is detected by the lower PMT only (non-coincidence counts). Counts registered below a certain threshold (channel 150) are discarded because these include background. In ParaLux high efficiency mode, the contents of both spectra are added together.

Traditionally ^{14}C, ^{35}S, ^{33}P and ^{45}Ca have been the isotopes used in Cytostar-T applications. Applications for the shorter path length isotopes have not been developed to such an extent due to their lower efficiency of counting on the Cytostar-T plate. The introduction of new count modes offers an opportunity to assess applications based on ^{125}I and ^{3}H for Cytostar-T assays.

Results

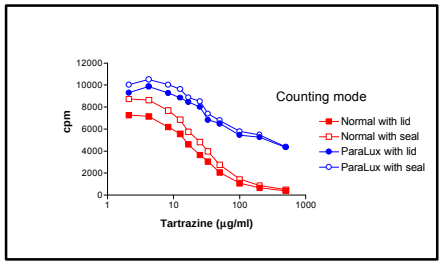

Figure 2. The effect of ParaLux counting on Cytostar-T colour quench. [^{125}I]Streptavidin was formalin fixed to Cytostar-T plate wells. Increasing tartrazine concentrations were added to the wells and the plate counted in the MicroBeta with either the lid or a plate seal in normal or high efficiency mode. Single well values (n=2).

In Figure 2, the ability of the ParaLux circuitry to deal with coloured samples is demonstrated. The replacement of the Cytostar-T lid with a plate seal yielded a slight increase in counts but did not significantly improve the ability of the system to count coloured samples. Therefore, colour quench correction is still required.

Figure 3 shows a receptor binding assay on the Cytostar-T plates. This system is potentially suitable for carrying out ligand binding assays in an homogeneous format, without disturbing the equilibrium between bound and free ligand, and while maintaining the cells in a more physiological environment.

In this example, ParaLux counting yielded an increase in counts compared to the normal counting mode.

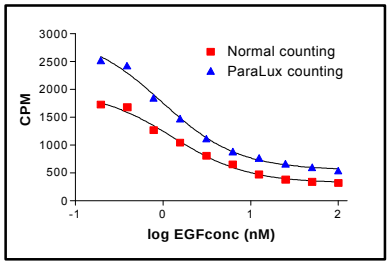

Figure 3. Competition binding of [^{125}I]EGF to EGF-Rs expressed on A431 cells measured by Cytostar-T binding. 80% confluent A431 cell monolayers were incubated at 37°C for two hours in assay buffer (20mM Hepes, pH 7.5, 2mM $CaCl_2$, 0.1% (w/v) BSA) containing [^{125}I]EGF (300pM final concentration) before counting in the MicroBeta. ParaLux counting was conducted in high efficiency mode. Triplicate data points +/-SEM (n=3).

Figure 4 shows the potential for higher counts with tritium in the TopCount using HECM. In addition, the replacement of the Cytostar-T lid with a plate seal further increased the counts. This strategy may be suitable for stopped assays or assays to which further additions need not be made.

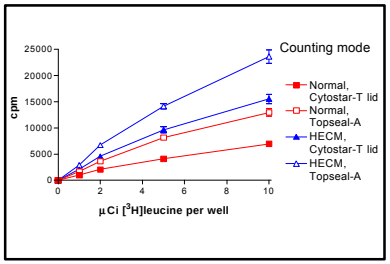

Figure 4. [^{3}H]Leucine uptake in V79 cells. Cells were plated at 4x10^{4} per well and incubated overnight in leucine free medium with tritiated leucine added at the activities indicated. The plate was counted in the TopCount in HECM with the window set at 5-256 and with a white card placed underneath the plate. Values are means (n=2).

Arachidonic acid release on the Cytostar-T plates has previously been demonstrated[4]. Figure 5 shows the utility of the TopCount HECM in an arachidonic acid release assay using [^{3}H]arachidonic acid.

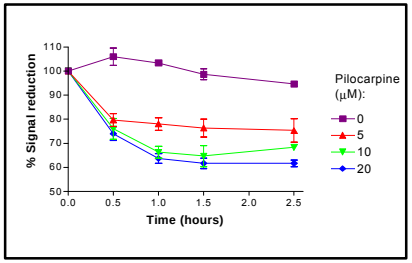

Figure 5. [^{3}H]Arachidonic acid release from CHOM1 cells. Cells (2x10^{4}/well) were incubated overnight with 0.5mCi arachidonic acid. The label was removed and the cells incubated for 2.5 hrs with agonist and BSA (2% w/v). The plate was counted at frequent intervals in the TopCount using HECM. Triplicate data points +/-SEM (n=3).

Discussion

New counting modes on both the MicroBeta and TopCount have been used to examine Cytostar-T applications. The new modes can be used to increase counts and have been used in this study with ^{3}H and ^{125}I, isotopes not traditionally associated with Cytostar-T.

References

1. Amersham Biosciences Proximity News, No. 30.
2. Wallac, MicroBeta TriLux Application Note, May 1997.
3. TopCount Topics, TCA-029.
4. Amersham Biosciences Proximity News, No. C5.

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