

Application Notes

ScintiPlate® for solid-phase binding assays

INTRODUCTION

The ScintiPlate* is a 96-well polystyrene microplate designed for solid phase radiolabel binding assays.

The ScintiPlate comprises clear polystyrene wells that are permanently fixed in an opaque white matrix.

When the plate is formed, scintillator is incorporated throughout the wells. In this way the scintillator becomes an integral part of the plastic well. Consequently there is no possibility that it can either detach from the well or interfere with the binding properties of the polystyrene, which is subsequently treated to impart superior protein-binding characteristics.

ScintiPlate wells are formed entirely of clear plastic. This has numerous advantages over plates comprised of white-sided wells:

- Light is transmitted much more efficiently in clear plastic, which results in higher counting efficiency.
- White pigment, that adversely affects binding capacity, is absent.
- Optimum counting geometry can be achieved. The preferred method for scintillation counting employs two photomultiplier tubes in each detector. Figure 1 illustrates counting geometry in the MicroBeta®.
- Light is transmitted towards the detectors through the clear plastic and the effects of colour quench are thereby minimized.
- Cells can be inspected with a microscope.

The white matrix that completely surrounds each well eliminates optical crosstalk and thereby maintains the highest possible counting efficiency.

ScintiPlate is a non-destructive method because no liquid scintillation cocktail is required. Radioactively labelled molecules that are bound to the well induce scintillation events in the plastic.

With fewer assay steps necessary and the capacity to study reactions kinetically, the ScintiPlate together with MicroBeta TriLux or MicroBeta JET, makes possible the development of many new assay types.

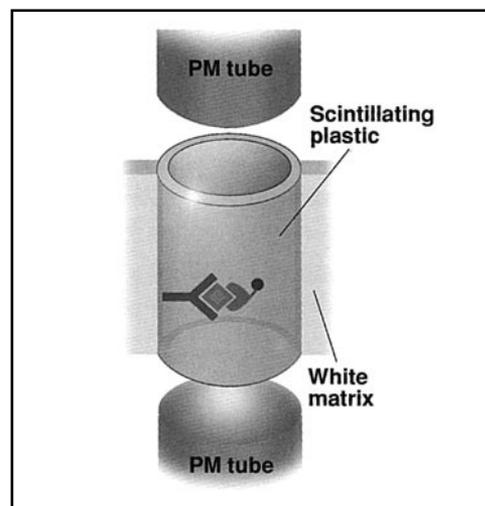


Figure 1. MicroBeta and ScintiPlate counting geometry

* patent applied

SCINTIPLATE PRODUCTS

There are three ScintiPlate products.

1450-501 ScintiPlate (25)

1450-502 ScintiPlate (100)

1450-551 ScintiPlate SA (10)
Streptavidin coated

The streptavidin binding capacity (6.0×10^6 moles biotin) is maximized by attaching the protein to the plate using a covalent binding technique.

Other ScintiPlate™ coatings are available on request. Examples include anti-Mouse IgG, anti-rabbit IgG and poly-lysine.

SCINTIPLATE APPLICATIONS BY MICROBETA

The ScintiPlate can be used in all solid phase binding assays where the label is a radioactive isotope. Examples of some typical binding assays are listed below.

IRMA sandwich assays - e.g. T4 competitive immunoassay

RIA competitive assays - e.g. estradiol

Coated receptor assays - e.g. cloned G-protein or fast ion-receptor assays

Hybridization assays - e.g. DNA/RNA hybridization or detection of point mutations

Enzyme activity assays - e.g. kinase with biotinylated substrate

Cell binding studies - e.g. 2-site antibody to cell-surface receptors

Binding experiments involving a labelled biotinylated compound - e.g. biotinylated substrate

Kinetic assays involving a tritiated compound - e.g. an antibody-antigen or receptor-binding reaction.

In the following section three applications of the ScintiPlate are illustrated in detail: estradiol radioimmunoassay, T4 RIA and binding of a biotinylated oligonucleotide.

Estradiol RIA:

Estradiol measurement was performed as a solid-phase RIA using ^3H -labelled estradiol. The reference DELFIA® Estradiol kit (Wallac, 1244-056) was made according to the kit instructions.

Procedure: A ScintiPlate was coated with anti-rabbit IgG ($1.5 \mu\text{g}/\text{well}$). For the sample preparation, danazol was diluted to a concentration of $5 \mu\text{g}/\text{mL}$ in assay buffer. $25 \mu\text{L}$ of standards, samples and controls and $100 \mu\text{L}$ estradiol antiserum (concentration of $0.06 \mu\text{g}/\text{mL}$, dilution in assay buffer) were pipetted in triplicate into the pre-washed ScintiPlate. The plate was incubated for 30 minutes and $100 \mu\text{L}$ of ^3H -estradiol dilution was added to the wells ($1\text{-}10 \text{ pmol}/\text{L}$). The ScintiPlate was incubated for a further 2 hours and the wells were washed four times with an automatic DELFIA PlateWash using Wash Solution (Wallac Oy). The ScintiPlate was dried for an hour at RT, before measurement with MicroBeta (counting time 3 minutes).

Results: The best signal-to-noise ratio was found to be at $1 \text{ pmol}/\text{L}$ of ^3H -estradiol. The coefficient of variation (CV%) varied from 0.4% to 11.7% (figure 2).

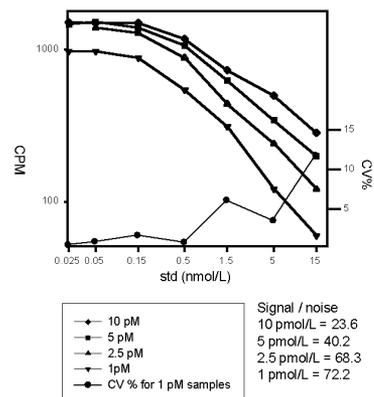


Figure 2. RIA Estradiol measurement with dilution series of ^3H -labelled estradiol, and CV % for the standards with $1 \text{ pmol}/\text{L}$ of labelled estradiol.

The correlation between the RIA and DELFIA methods is shown in figure 3.

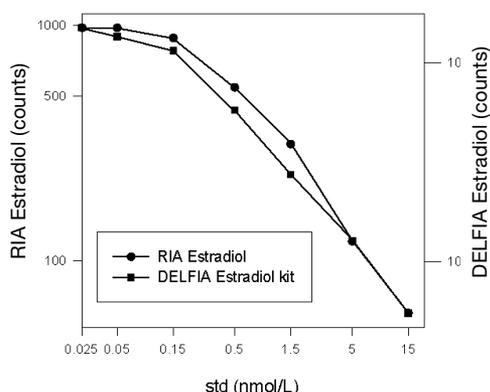


Figure 3. RIA Estradiol vs. Estradiol DELFIA kit.

T4 RIA:

T4 measurement was performed as a solid-phase competitive RIA. ^{125}I -labelled streptavidin was used as a label. The reference T4 DELFIA kit (Wallac) was done according to the kit instructions.

Procedure: Biotinylated T4 was diluted to achieve a concentration of 600 nmol/L. The solution, containing 60 μL biotinylated T4 -dilution, 60 μL antibody stock solution (Wallac, concentration 12 $\mu\text{g}/\text{mL}$) and 6 mL T4 buffer was prepared. 25 μL of standards were pipetted as duplicates to the pre-washed wells and 200 μL of previously prepared solution was added to the wells. The ScintiPlate was incubated for 90 minutes. The plate was then washed four times with DELFIA PlateWash and Wash Solution (Wallac) and a dilution of ^{125}I -Streptavidin was made to achieve a concentration of 100,000 DPM. 200 μL of the ^{125}I -Streptavidin -dilution was added to the wells and the plate was incubated for an hour. Finally the plate was washed six times. The plate was dried for an hour at RT before measurement with MicroBeta (counting time 3 minutes).

Results: The coefficient of variation (CV%) varied from 1.1 to 13.6. The linear area of the standard curve was between 20-300 nmol/L (figure 4).

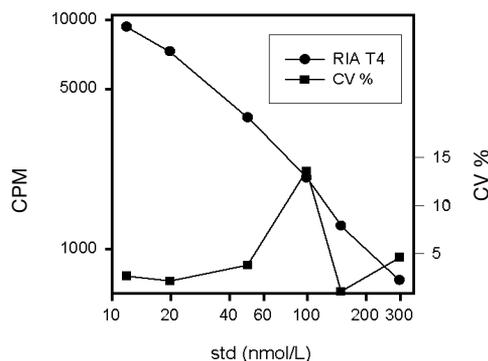


Figure 4. RIA T4 and CV %.

The correlation between T4 RIA and DELFIA, T4 analyses is seen in figure 5.

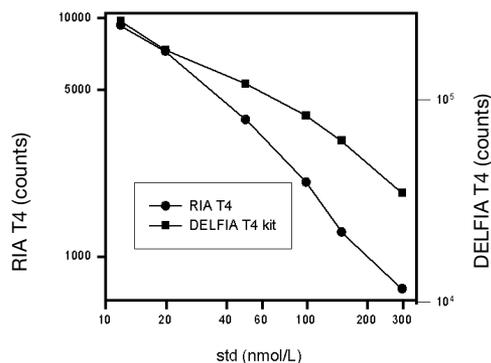


Figure 5. Solid phase RIA T4 vs DELFIA T4 kit.

The enzyme activity assay:

An enzyme activity assay was performed using a kinase and its biotinylated substrates. The effects of two concentrations of substrate, a) 3 and b) 6 μM were tested.

Procedure: The enzymatic reaction took place in Eppendorf tubes. The reactions contained 0.25 μg enzyme, 3 or 6 μM substrate, 1 mM DTT, 10 mM MgCl_2 , 50 mM HEPES pH 6.8, 0.015 % Brij35, 2.5% DMSO and 50 mM ATP + 92 nM $[\gamma\text{-}^{33}\text{P}]$ -ATP in a total volume of 25 μL . The reactions were incubated at +30 $^\circ\text{C}$ for two hours. After the incubation the reactions were diluted a) 1:100 and b) 1:200 to obtain a substrate concentration of 30 nM. Diluted reactions were pipetted in triplicate into pre-washed Streptavidin

coated ScintiPlate. The plate was incubated with slow shaking for half an hour. After incubation the ScintiPlate was measured with MicroBeta using a default ³³P counting protocol.

Results:

Table 1 shows the signal and background values for reactions containing 3 or 6 µM substrate. In this case, an adequate signal-to-noise ratio is obtained with the lower substrate concentration.

[S]	signal	noise	S:N
3 µM	511	12	43
6 µM	763	15	51

Table 1. The signal-to-noise ratio with two concentrations of substrate.

OPTIMIZATION OF COUNTING

ScintiPlate assays, like all assays, must be optimized to get the best results. Optimization concerns the bio-reaction part of coating as well as counting in the MicroBeta.

Coating optimization

Coating of the ScintiPlate is the same as all other plate coatings. First, the protein to be coated is diluted to a concentration of 0.5-10 µg/mL in, for example, phosphate buffer. The plates are incubated overnight and then washed. All the remaining binding sites can be blocked (saturated) by dispensing, for example, 0,5 % bovine serum albumin into the wells and by incubating the wells for a few hours at RT. The exact coating procedure must be fully optimized for each coating. The following factors should be considered.

- amount of protein per well
- pH of the coating buffer
- ionic strength of the buffer
- coating and saturation times
- incubation temperature
- number of washing cycles after coating and saturation

The stability and reproducibility of the coating will also depend on the protein structure. Usually, antibody coatings are quite stable whereas other smaller recombinant proteins may need special stabilizing treatments.

Once the optimum coating procedure for the ScintiPlate has been determined, the immuno-reaction parameters that are listed below should be considered.

- time
- temperature
- buffer
- assay volume

Typical buffers that are used include TRIS-HCl and PBS. Also, DELFIA Assay Buffer, which contains appropriate additives and preservatives, has been optimized for a broad range of immunoassays.

The use of extreme conditions, such as high / low pH or high detergent concentrations should be avoided.

Optimization of counting performance

The best counting performance is obtained if the ScintiPlate is washed and dried after the incubation phase.

The wash step will reduce non-specific binding and also eliminate counting efficiency variations caused by different coloured compounds.

Tritium and other weak beta emitters can be partially absorbed or deflected by any residual water or buffer solution that remains on the well surface after washing. To avoid this the plates can be dried in an oven at 37°C or left for 1 to 3 hours at RT.

COUNTING GEOMETRY OF SOLID PHASE BINDING ASSAYS

Radioactive labels bound to a microplate or a bead exhibit “ 2π ” counting geometry regardless of whether liquid or solid scintillation counting is performed. In other words, as the isotope decays, the emitted beta particle can move in any direction in the “ 4π ” solid angle that surrounds the labelled compound. If the labelled compound is attached to a surface, only a half of the total events can be detected.

In the case of the ScintiPlate or a scintillating bead, only beta particles that move into the wall or the bead produce scintillation events.

(Conversely, in the case of a coated non-scintillating plate assay, only beta particles that move into the cocktail will produce scintillation events.)

Thus the counting efficiency of solid-phase binding assays have systematically lower counting efficiencies compared to assays that have the labelled compounds fully dissolved in liquid cocktail.

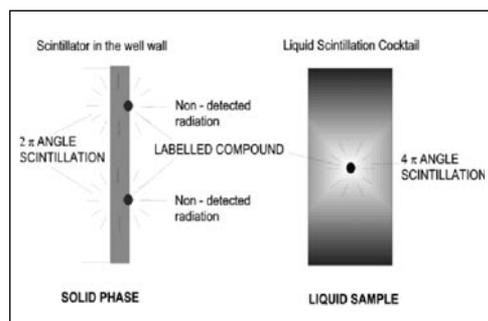


Figure 6. Counting geometry for solid- phase counting compared to liquid samples.

Isotope	Counting efficiency, %
^3H	30
^{125}I	61
^{33}P	60

Table 2. Typical ScintiPlate counting efficiencies.

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