Amersham
Yttrium Silicate (YSi) SPA
Reagents, Type 1
for homogeneous RIA

Product Booklet

Codes:  Anti-rabbit YSi SPA Beads RPN140
        Anti-mouse YSi SPA Beads RPN141
        Anti-sheep YSi SPA Beads RPN142
        Protein A YSi SPA Beads RPN143
Page finder

1. Legal 3
2. Handling 4
   2.1. Safety warnings and precautions 4
   2.2. Storage 4
   2.3. Expiry 4
3. Description 5
4. Introduction 6
5. Background to scintillation proximity assay 7
6. Summary of scintillation proximity assay for RIA 8
7. Assay methodology 9
   7.1. Materials and equipment required 9
   7.2. Reagent preparation 9
   7.3. Assay protocols 10
   7.4. Representative protocols 11
   7.5. Calculation of results 16
8. Developing a scintillation proximity assay 18
   8.1. Choice of scintillation proximity assay reagent 18
   8.2. How much reagent to add 19
   8.3. Choice of tracer 19
   8.4. Tracer concentration 19
   8.5. Incubation time 20
   8.6. Incubation temperature 20
   8.7. Counting 20
   8.8. Assay tubes 23
9. Troubleshooting guide 24
10. References 25
11. Related products 26
1. Legal

GE, imagination at work and GE monogram are trademarks of General Electric Company.

All third party trademarks are the property of their respective owners.

© 2007 General Electric Company – All rights reserved.

Previously published 1992

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available on request. Contact your local GE Healthcare representative for the most current information.

http://www.gehealthcare.com/lifesciences

GE Healthcare UK Limited.
Amersham Place,
Little Chalfont, Buckinghamshire,
HP7 9NA, UK
2. Handling

2.1. Safety warnings and precautions

**Warning:** *For research use only.* Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

**Caution:** For use with radioactive material.

This product is to be used with radioactive material. Please follow the manufacturer’s instructions relating to the handling, use, storage and disposal of such material. All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.

2.2. Storage

Store at 2–8°C.

2.3. Expiry

The expiry date is stated on the package and will be at least four weeks from the date of despatch.
3. Description

These generic products have been designed to enable researchers to convert existing heterogeneous assays to homogeneous scintillation proximity assay systems. A range of generic reagents, consisting of second antibodies or protein A coupled to fluomicrospheres is provided lyophilized. Upon reconstitution, 100 μl/tube scintillation proximity assay reagent is optimal for assay performance in most assays.

Each bottle of reagent contains sufficient material for 500 tubes in a typical assay.
- No separation step
- No liquid scintillant
- Automatable
- One tube assay
- Generic reagents
4. Introduction

Scintillation proximity assay (SPA) is a novel technique applicable to radiobinding assays which eliminates the need for a separation step and addition of liquid scintillant.

Antibody bound ligand is reacted with a scintillation proximity assay reagent which contains either second antibody or protein A bound to fluomicrospheres. Any radiolabelled ligand that is bound to the primary antibody is immobilized on the scintillation proximity assay fluomicrosphere which will produce light. Measurement in a \( \beta \) scintillation counter enables the amount of radiolabelled ligand bound to be calculated.

The availability of this range of generic scintillation proximity assay fluomicrospheres allows the simple conversion of existing heterogeneous assay systems into the convenient scintillation proximity assay format.

Generic scintillation proximity assay reagents are available coupled to anti-rabbit, anti-mouse and anti-sheep IgG as well as protein A, making this new technology suitable for a large range of assays.

**Anti-rabbit YSI SPA Beads, RPN140**
For use when the primary antibody is of rabbit origin.

**Anti-mouse YSI SPA Beads, RPN141**
For use when the primary antibody is of mouse origin; particularly suited to assays using monoclonal antibodies.

**Anti-sheep YSI SPA Beads, RPN142**
For use when the primary antibody is of sheep or goat origin.

**Protein A YSI SPA Beads, RPN143**
For use when the primary antibody is of rabbit, mouse or guinea pig origin.
5. Background to scintillation proximity assay

In an aqueous environment relatively weak β-emitters, notably $[^3H]$ and $[^{125}I]$ (Auger electrons), need to be close too scintillant molecules in order to produce light, otherwise the energy is dissipated and lost in the solvent. GE Healthcare has used this concept to develop a range of homogeneous assays by coupling specific antibodies on to fluomicrospheres (beads containing scintillant) whereby:

- No separation of bound from free is required – only radioactive ligand which is bound to the antibody gives a signal
- No use of liquid scintillant is required – the scintillant is incorporated chemically within the microsphere
- Complete automation of the assay is possible – simply pipette the reagents into a minicounting vial, allow equilibrium to be reached and count.

This technique which is termed scintillation proximity assay (1–7) is suited to radioligand and binding assays, particularly radiimmunoassay.
6. Summary of scintillation proximity assay for RIA

In common with conventional heterogeneous radioimmunoassay systems, the assay is based on the competition between unlabelled ligand and a fixed quantity of radiolabelled ligand for a limited number of binding sites on a specific antibody. With fixed amounts of antibody and radioactive ligand the amount of radioactive ligand bound by the antibody will be inversely proportional to the concentration of added non-radioactive ligand.

In GE Healthcare’s scintillation proximity assays (SPA) the antibody bound ligand is reacted with a scintillation proximity assay reagent, which contains either a second antibody or protein A that is bound to fluomicrospheres. Any radiolabelled ligand that is bound to be the primary antibody will therefore be immobilized on the fluomicrospheres which will produce light.

Measurement in a β scintillation counter enables the amount of labelled ligand to be calculated. The concentration of unlabelled ligand in a sample is then determined by interpolation from a standard curve.

**Figure 1**

Light if L* bound

\[ F \xrightarrow{Ab + (LorL*)} AbL or AbL* \]

F = fluomicrosphere with protein A or second antibody
Ab = primary antibody, L* = labelled ligand, L = unlabelled ligand
7. Assay methodology

Users are recommended to read this entire section before starting work.

7.1. Materials and equipment required

The following are required but not provided:
- Disposable polypropylene, polystyrene or glass tubes
- Assays should be performed in tubes which are suitable for direct counting in a β scintillation counter. Miniscintillation vials are available from supplier such as Packard and Beckman instruments. For example Bio-Vial™ (13 x 55 mm)
- Pipettes (100 μl of scintillation proximity assay reagent should be sufficient for most assays)
- Test tube rack
- Vortex mixer
- Orbital shaker
- Magnetic stirrer
- β scintillation counter

7.2. Reagent preparation

Reconstitute the bottle containing scintillation proximity assay reagent with 50 ml assay buffer. Gently shake the bottle for 5 minutes to dissolve the buffer salts.

Note: The scintillation proximity assay fluomicrosphres are insoluble. The bottle contents should be magnetically stirred to ensure a homogenous suspension when pipetting into assay tubes/wells.

Storage: After reconstitution the scintillation proximity assay reagent will be stable for up to 7 days stored at 2-8°C. For extended storage, store at -15°C to -30°C.
7.3. Assay protocols

Assays may be performed in one of two ways as shown below:

I) Same day protocol
II) Overnight protocol

The choice will be determined by the individual assay reagents, conditions and laboratory convenience. In general, equilibrium is reached faster if standard, tracer and first antibody are incubated prior to scintillation proximity assay reagent addition. This allows same day counting in many assays.

Speed of reaction is markedly increased if tubes containing scintillation proximity assay reagent are agitated to keep fluomicrospheres in suspension. Orbital shakers are strongly recommended.
7.4. Representative protocol

These protocols were originally developed and optimized for GE Healthcare’s thromboxane B2 and 6-Keto-prostaglandin F1α assays. The incubation times are given for guidance only and may need optimizing for different assays.

One day protocol
1. Label suitable polypropylene assay tubes (see materials section page 9) for non-specific binding (NSB), zero standard (B₀), standards and sample tubes.
2. Pipette 200 μl of assay buffer into the NSB tubes.
3. Pipette 100 μl of assay buffer into the B₀ tubes.
4. Pipette 100 μl of each standard into the appropriately labelled tubes.
5. Pipette 100 μl of the samples to be assayed into the appropriately labelled tubes.
6. Pipette 100 μl of tracer into all tubes.
7. Pipette 100 μl of antiserum into the B₀, standard and sample tubes.
8. Mix the contents of the tubes with a vortex mixer and incubate for 2 hours at room temperature (15–30°C). The time of incubation will be different for each assay and will therefore need optimizing.
9. Place the scintillation proximity assay reagent on to a magnetic stirrer and adjust the stirring speed to ensure a homogeneous suspension. Then add 100 μl to all the tubes.
10. Cap the tubes and mix on an orbital shaker for 4 hours at room temperature (15–30°C). The shaking speed should be optimized for individual shakers. 200 rpm is sufficient for most orbital shakers.
11. Determine the amount of tracer bound to the bead by counting the tubes in a β scintillation counter.
Overnight protocol

1. Label suitable polypropylene assay tubes (see materials section page 9) for non-specific binding (NSB), zero standard (B₀), standards and sample tubes.
2. Pipette 200 μl assay buffer into the NSB tubes.
3. Pipette 100 μl of assay buffer into the B₀ tubes.
4. Pipette 100 μl of each standard into the appropriately labelled tubes.
5. Pipette 100 μl of the samples to be assayed into the appropriately labelled tubes.
6. Pipette 100 μl of tracer (10 000 cpm) into all tubes. Either tritium or iodine-125 tracers can be used.
7. Pipette 100 μl of antiserum into the B₀, standard and sample tubes.
8. Place the appropriate SPA-reagent on to a magnetic stirrer and adjust the stirring speed to ensure a homogeneous suspension. Then add 100 μl to all tubes.
9. All tubes should now contain a total volume of 400 μl.
10. Cap the tubes and mix on an orbital shaker for 15–20 hours at room temperature (15–30°C). The shaking speed should be optimized for individual shakers. 200 rpm is sufficient for most orbital shakers.
11. Determine the amount of tracer bound to the bead by counting the tubes in a β scintillation counter. See section on counting (page 20).

Note:

1. Scintillant should not be added to the tubes. If the scintillation counter has removable racks compatible with the assay tubes, tubes can be loaded directly into the racks. Alternatively assay tubes can be placed into standard liquid scintillation vials and loaded into the counter.
Table 1. Overnight protocol (all volumes in microlitres)

<table>
<thead>
<tr>
<th>Tube</th>
<th>NSB</th>
<th>Zero standard</th>
<th>Standards</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>200</td>
<td>100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tracer</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Standards</td>
<td>–</td>
<td>–</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>Samples</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Antiserum</td>
<td>–</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Scintillation proximity assay reagent</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Cap, shake overnight and count

Table 2. One day protocol (all volumes in microlitres)

<table>
<thead>
<tr>
<th>Tube</th>
<th>NSB</th>
<th>Zero standard</th>
<th>Standards</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>200</td>
<td>100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tracer</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Standards</td>
<td>–</td>
<td>–</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>Samples</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Antiserum</td>
<td>–</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Scintillation proximity assay reagent</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Mix and incubate for 2 hours

Cap, shake for 4 hours and count
Table 3. Protocol for 96 well sample plates [all volumes are in microlitres]

<table>
<thead>
<tr>
<th>Tube</th>
<th>NSB</th>
<th>Zero standard</th>
<th>Standards</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>100</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tracer</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Standards</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Samples</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>Antiserum</td>
<td>-</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Scintillation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximity assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent, Type I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reconstituted with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 ml assay buffer</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Seal plates, shake overnight and count
Determination of total activity and percent binding (optional)

It is not convenient to routinely determine the total counts and percent binding in scintillation proximity assays. It is recommended that $B_0$ cpm values are recorded in successive assays in order to monitor assay performance.

GE Healthcare's quality control laboratories routinely test the total counts and percent binding parameters in set assays with all new batches of scintillation proximity assay reagents and the products are not released unless specifications are met.

Nevertheless if customers wish to determine the total counts and percent binding in an assay, the following protocol is recommended.

1. Label in duplicate tubes for total counts (TC) and $B_0$.
2. Pipette 200 μl of assay buffer into the TC tubes.
3. Pipette 100 μl of assay buffer into the $B_0$ tubes.
4. Pipette 100 μl of tracer into all tubes.
5. Pipette 100 μl of antiserum into all tubes.
6. Pipette 100 μl of scintillation proximity assay reagent into the $B_0$ tubes.
7. Cap the tubes and mix on an orbital shaker for 15–20 hours at room temperature (15–30°C). The shaking speed should be optimized for individual shakers. 200 rpm is sufficient for most orbital shakers.
8. Centrifuge all tubes at 2500 x g for 10 minutes at 4°C.
9. Decant the supernatant into suitably labelled scintillation vials.
10. Add 10 ml of an appropriate scintillant. Cap, mix and determine the radioactivity by counting in a β scintillation counter.

**Note:** This protocol describes the determination of total activity and percent binding in the overnight assay. If the one day protocol is used the incubation times should be changed accordingly.
7.5. Calculation of results

Calculation of standard curve data
The assay data collected should be similar to the data shown in table 3.

i) Calculate the average counts per minute (cpm) for each set of replicate tubes.

ii) Calculate the percent bound for each standard and sample using the following relationship.

\[
\% B/B_0 = \frac{(\text{standard or sample cpm} - \text{NSB cpm}) \times 100}{(B_0 \text{ cpm} - \text{NSB cpm})}
\]

A standard curve may be generated by plotting the percent B/B_0 as a function of the log standard concentration.

Plot % B/B_0 (y axis) against pg standard per tube (x axis) as shown in figure 2. The pg/tube value of the samples can be read directly from the graph.

Calculation of total counts and percent binding

\[
\text{percent Binding} = 100 - \frac{B_0 \text{ cpm}^* \times 100}{10 \text{ cpm}^*}
\]

*Data obtained from optional protocol, page 13.
Table 4. Typical assay data

<table>
<thead>
<tr>
<th>Standard</th>
<th>pg</th>
<th>Average cpm*</th>
<th>B/B₀ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSB</td>
<td>–</td>
<td>25</td>
<td>–</td>
</tr>
<tr>
<td>O</td>
<td>0</td>
<td>3070</td>
<td>100</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>2447</td>
<td>79.7</td>
</tr>
<tr>
<td>D</td>
<td>13</td>
<td>1916</td>
<td>62.4</td>
</tr>
<tr>
<td>C</td>
<td>40</td>
<td>1228</td>
<td>40.0</td>
</tr>
<tr>
<td>B</td>
<td>110</td>
<td>623</td>
<td>20.3</td>
</tr>
<tr>
<td>A</td>
<td>300</td>
<td>285</td>
<td>9.2</td>
</tr>
</tbody>
</table>

This data is typical of GE Healthcare’s Thromboxane B₂ [³H] scintillation proximity assay (SPA) system.

Figure 2. Typical standard curve
8. Developing a scintillation proximity assay

GE Healthcare's second antibody and protein A scintillation proximity assay products are provided for researchers who wish to convert their own assays to SPA.

The following factors should be considered when incorporating scintillation proximity technology into existing assays.

8.1. Choice of scintillation proximity assay reagent

The table below describes which scintillation proximity assay reagents are suitable for different sources of primary antibody (IgG).

The relative performance of anti-rabbit and protein A reagents in assays using rabbits derived polyclonal antibodies is determined by the characteristics of those individual assays.

<table>
<thead>
<tr>
<th>Source of primary antibody</th>
<th>Compatible scintillation proximity assay reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>i) Rabbit</td>
<td>anti-rabbit RPN140</td>
</tr>
<tr>
<td></td>
<td>* Protein A RPN143</td>
</tr>
<tr>
<td>ii) Mouse</td>
<td>anti-mouse RPN141</td>
</tr>
<tr>
<td></td>
<td>* Protein A RPN143</td>
</tr>
<tr>
<td>iii) Sheep</td>
<td>anti-Sheep RPN142</td>
</tr>
<tr>
<td>iv) Goat</td>
<td>anti-Sheep RPN142</td>
</tr>
<tr>
<td>v) Guinea pig</td>
<td>* Protein A RPN143</td>
</tr>
</tbody>
</table>

* Depending on the IgG subtype
8.2. How much reagent to add
The scintillation proximity assay generic reagents have been formulated in terms of fluomicrospheres and immunobinder concentration so that optimal performance is achieved with 100 μl additions.

In most assays a simple substitution of the separation reagent for 100 μl of the appropriate scintillation proximity assay reagent is all that is needed to convert an existing heterogeneous assay to a homogeneous scintillation proximity assay system.

However if the assay uses a high concentration of primary antibody it may be necessary to increase the amount of scintillation proximity assay reagent in order to saturate the primary antibody.

8.3. Choice of tracer
Scintillation proximity technology is suitable for detection of both tritium and iodine-125 tracers.

Note: High non-specific binding has been obtained with some high molecular weight molecules, such as some peptides. In such instances the scintillation proximity assay technology may not be suitable using this type of reagent.

8.4. Tracer concentration
The counting efficiency of fluomicrospheres is slightly lower than conventional liquid scintillants. Consequently cpm's/tube will be slightly lower with similar tracer concentrations.

In order to achieve equivalent counting precision, counting time should be increased to compensate, or the tracer concentration increased.

The total precision of scintillation proximity assays is however superior to heterogeneous assays due to the fewer manipulative processes and lack of centrifugation.
8.5. Incubation time
It is important that assay tubes are incubated until equilibrium is reached, otherwise assay drift may be experienced. Incubation times are typically between 2 and 6 hours depending on the assay systems being used.

8.6. Incubation temperature
Standard binding assay incubation temperatures are suitable for scintillation proximity assays. However, it is recommended where possible that incubation should be carried out at a temperature similar to that in a β scintillation counter, or to allow tubes to equilibrate in the counter before counting.

8.7. Counting
After incubation the tubes should be briefly shaken to resuspend the fluomicrospheres and counted in a β scintillation counter. It is important that the total counting period should be no longer than 3 hours, otherwise drift due to fluomicrosphere settling may be experienced.

If prolonged counting time is unavoidable the addition of 10% sucrose to the assay will minimize assay drift.

For counters fitted with spectrum analysis packages the suitable window opening should be determined and the machine set accordingly.

Windows for other counters should be set wide open for example:
\[\text{a) Beckman LS7800 where H\# is set at 0 and the windows open from 10-999.}\]
\[\text{b) Packard Tricarb 460 with window open from 0-999.}\]
\[\text{c) LKB 1209/1215 Rackbeta with window open from 5-999. SQP(I) and SQP(E) are not used.}\]
Automatic quench correction should not be used.

GE Healthcare provides two types of scintillation proximity fluomicrospheres supplied coated with second antibodies or protein A. Table 6 describes the major differences between type I and type II scintillation proximity fluomicrospheres.

Table 6.

<table>
<thead>
<tr>
<th></th>
<th>Type I SPA reagent</th>
<th>Type II SPA reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Automatable assays</td>
<td>Not easily</td>
<td>Readily automatable</td>
</tr>
<tr>
<td></td>
<td>automatable</td>
<td></td>
</tr>
<tr>
<td>Agitation during incubation</td>
<td>Required</td>
<td>Not required</td>
</tr>
<tr>
<td>Typical assay incubation time</td>
<td>6–18 hours</td>
<td>2–6 hours</td>
</tr>
<tr>
<td>Counting efficiency (9)</td>
<td>$^{3}$H 60%</td>
<td>$^{1}$H 40%</td>
</tr>
<tr>
<td></td>
<td>$^{125}$I 80%</td>
<td>$^{125}$I 40%</td>
</tr>
<tr>
<td>Non-specific binding</td>
<td>May be high with</td>
<td>low</td>
</tr>
<tr>
<td></td>
<td>high molecular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>weight ligands</td>
<td></td>
</tr>
<tr>
<td>Protocols for</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>microplate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>scintillation counters</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Instrument settings for microplate scintillation counters are described in the following tables.

**Table 7. Instrument settings for TopCount™**

<table>
<thead>
<tr>
<th>SPA reagent</th>
<th>Scintillator Type</th>
<th>Energy range</th>
<th>Efficiency mode</th>
<th>Isotope to be counted</th>
<th>Window settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>solid</td>
<td>low</td>
<td>normal</td>
<td>Tritium $^3$H</td>
<td>1.5–50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Iodine-125</td>
<td>1.5–100</td>
</tr>
<tr>
<td>Type II</td>
<td>liquid</td>
<td>low</td>
<td>high</td>
<td>Tritium $^3$H</td>
<td>0–50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Iodine-125</td>
<td>0–100</td>
</tr>
</tbody>
</table>

**Table 8. Window settings for Microbeta™ 1450**

<table>
<thead>
<tr>
<th>SPA reagent</th>
<th>Window settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>5–500</td>
</tr>
<tr>
<td>Type II</td>
<td>5–500</td>
</tr>
</tbody>
</table>
8.8. Assay tubes

Assays which are to be counted in conventional counters should be performed in miniscintillation vials which can be obtained from suppliers such as Packard and Beckman (for example, Bio-vial). These can be placed directly into the counting racks of the appropriate scintillation counter. Alternatively, stoppered glass, polypropylene or polystyrene tubes which fit into standard scintillation vials can be used.

Assays which are to be counted in microplate format counters should be performed in 96 well sample plates compatible with the instrument to be used. These are available from instrument suppliers.
9. Troubleshooting guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-NSB</td>
<td>• Reduce the amount of bead in the assay.</td>
</tr>
<tr>
<td></td>
<td>• Use type II fluomicrospheres. (See table 6).</td>
</tr>
<tr>
<td>No bound counts</td>
<td>• Check that the coated fluomicrospheres have been added to the assay.</td>
</tr>
<tr>
<td></td>
<td>• Check that the coated fluomicrosphere is compatible with the source of primary antibody. (See table 5).</td>
</tr>
<tr>
<td>Low bound counts</td>
<td>• Check window setting on counter. (See section on counting, page 20).</td>
</tr>
<tr>
<td></td>
<td>• Increase the tracer concentration.</td>
</tr>
<tr>
<td>Very high counts throughout the assay</td>
<td>• Check whether scintillant has been added to tubes. Scintillant is not required.</td>
</tr>
<tr>
<td></td>
<td>• Ensure Iodine-125 is counted in a $\beta$ counter.</td>
</tr>
<tr>
<td>Drift in assay</td>
<td>• Check whether equilibrium has been reached.</td>
</tr>
<tr>
<td></td>
<td>• Add 10% sucrose to assay to minimize drift (type II fluomicrospheres only).</td>
</tr>
<tr>
<td>Unacceptable error between replicates</td>
<td>• Ensure fluomicrospheres are in homogeneous suspension before pipetting.</td>
</tr>
</tbody>
</table>
10. References

2. Immediate ligand detection assay, European Patent number 0154734.
11. Related products

Anti-rabbit PVT SPA Beads RPNQ0016
Anti-mouse PVT SPA Beads RPNQ0017
Anti-sheep PVT SPA Beads RPNQ0018
Protein A PVT SPA Beads RPNQ0019

GE Healthcare also provides a range of assay kits incorporating scintillation proximity assay technology:
cAMP $^{125}$I scintillation proximity assay (SPA) system RPA 538
cGMP $^{125}$I scintillation proximity assay (SPA) system RPA 540