

New bead types for SPA Imaging receptor binding assays

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Introduction

Recently, we have developed several new bead types that extend the range of ligands that can be used in SPA Imaging by providing alternative surfaces or augmented coatings. These additionally help to reduce non-specific background (NSB) which is often a major consideration in the success, or otherwise, of a RBA. SPA Imaging assays are reliant on being able to successfully couple one assay component to the SPA Imaging bead, in order to generate a signal. In this poster we look at some of the parameters of assay development and demonstrate the range of data that can be generated with SPA Imaging receptor binding assays (RBA's).

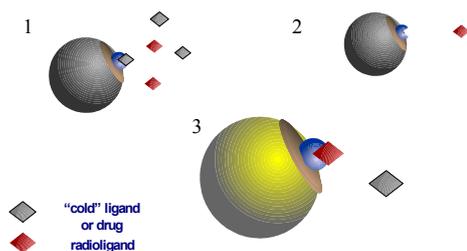


Figure 1. Schematic representation of receptor binding SPA imaging assay principle (1) Cold ligand or compound binds to the receptor captured onto the SPA imaging bead. No signal is generated. (2) No radioligand binds to the receptor: no signal is generated. (3) Radioligand binds to the receptor captured onto the SPA imaging bead bringing the radioisotope into close proximity with the scintillant within the bead, stimulating the bead to emit light.

Benefits of new beads

The new bead coatings broaden the range available for SPA Imaging RBAs. The addition of a PEI coating either after (Type A) or before (Type B) the WGA coating adds a positively charged surface to the bead which helps to reduce NSB from hydrophobic ligands. Both the PEI and polylysine coatings function in RBA's by binding membrane in a charge dependent manner. The positively charged surface captures the membrane by interacting with the negatively charged phospholipid head groups, useful for cell lines that have low surface glycosylation, such as insect cells. They are also useful in situations when the ligand of choice is glycosylated and thus unsuitable for use with WGA coated beads.

The new bead types described here include: polyethyleneimine (PEI) treated WGA-coated beads, (Type A, RPNQ0286/0287; Type B, RPNQ0288/0289); beads coated solely with PEI (RPNQ0098/0297); beads coated with polylysine (RPNQ0294/0295) and WGA-coated beads utilising an yttrium oxide (YOx) core (RPNQ0270/0272). The beads are also available in a Select-a-bead pack (RPNQ0291) which is designed to enable users to quickly identify the most appropriate bead type for their application.

Assay design

SPA Imaging beads are stable in a wide range of biological buffers, and over a broad pH range, so configuring assays is very simple. One of the key choices is exactly which bead type to use for the receptor-ligand combination under investigation. SPA Imaging beads offer the opportunity for testing several different bead types in parallel, using a single assay plate.

Figure 2 shows some example data for the selection of the best bead type, in this case for a Neuromedin U RBA. All bead types were tested on a single 384 well plate. With the data obtained, users can then determine which bead type is the most useful based on criteria such as signal window or signal to background ratio. All other assay parameters such as membrane mass,

bead mass and bead:receptor ratio can also be determined in single plate matrix experiments.

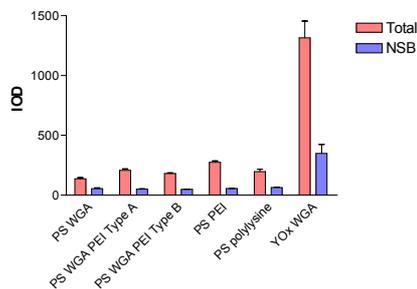


Figure 2. Bead selection test using the Imaging Bead Select-a-Bead kit. The data shown utilizes [¹²⁵I]Neuromedin U25 (Amersham Biosciences, IM349) binding to recombinant Neuromedin U receptors (Euroscreen, ES-450-M). The assays were imaged for 5 minutes, 3x3 binning with coincident averaging on the Amersham Biosciences LEADseeker™ Multimodality Imaging System (n = 3).

Assay validation

Having established the basic assay parameters, assay performance can be established as part of assay validation.

For example, it is important in RBAs to use a ligand concentration below the K_d. The SPA Imaging assay can be used to determine this parameter by means of a conventional saturation binding curve. All assay parameters are kept constant, except for the radioligand concentration, which is increased over a concentration range. Figure 3 shows some example data, using a Melanocortin 4 RBA, Figure 4 shows similar data, using a Neuromedin U RBA.

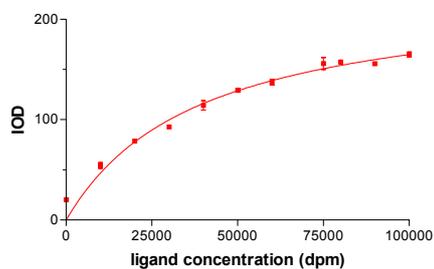


Figure 3. Saturation binding curve. The data shown utilizes [¹²⁵I][Tyr²][Nle⁴-D-Phe⁷]α-melanocyte stimulating hormone (Amersham Biosciences IM316) binding to Human Melanocortin 4 receptor (RBHMC4M, Receptor Biology, Perkin Elmer), captured on PS WGA-coated PEI treated Type A SPA Imaging beads. K_d was determined as 174.6pM, by back calculation from the dpm values, against a published filtration assay K_d of 190pM (n = 3).

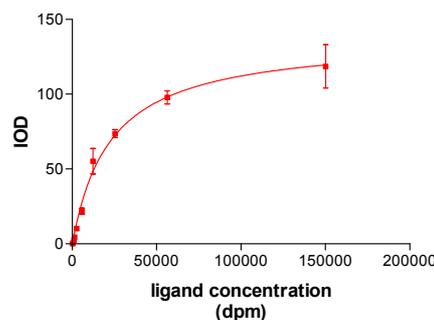


Figure 4. Saturation binding curve. The data shown

utilizes [¹²⁵I]Neuromedin U25 (Amersham Biosciences, IM349) binding to recombinant Neuromedin U receptors (Euroscreen, ES-450-M). Bead type was PS PEI SPA Imaging bead. K_d was determined as 103pM against a published filtration assay K_d of 50pM (n = 3).

Further validation can be carried out using other known ligands to generate competition binding curves. The examples below use the unlabelled ligand as a competitor.

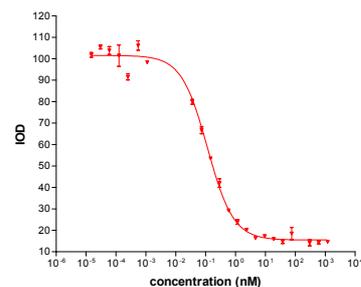


Figure 5. Competition binding curve. Assay system as shown in Figure 3. Bead type was PS WGA-coated, PEI treated Type A. Competition binding against unlabelled NDPαMSH. IC₅₀ 114.9pM (95% confidence limits 101.8 to 130.2pM) (n = 3).

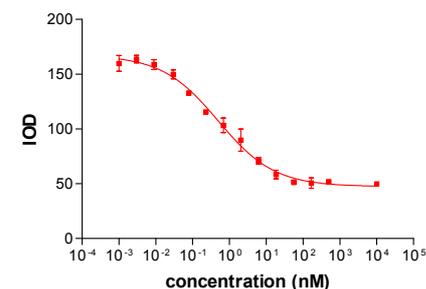


Figure 6. Competition binding curve. Assay system as shown in Figure 4. Competition binding against unlabelled Neuromedin U25, receptor captured on PS PEI SPA Imaging beads. IC₅₀ 497.2pM (95% confidence limits 317 to 779pM) (n=3).

The range of SPA Imaging beads, coupled with the extensive information available in pack leaflets and on the Amersham Biosciences web site, gives the user all the tools required for the rapid, successful development of Imaging RBA's.

CONCLUSION

- Amersham Biosciences now offers an enlarged range of SPA Imaging bead types suitable for receptor binding assays.
- The new range of bead coatings expands the range of both receptor sources and ligands that can be successfully used in SPA Imaging RBAs.
- SPA Imaging offers a rapid, simple, cheap single plate format for the optimisation of RBA's.
- The SPA Imaging format provides kinetically competent data.