TECHNICAL DATA SHEET

SPA Beads

Research Use Only. Not for use in diagnostic procedures.

PVT Copper His-Tag SPA Beads

Product Numbers: RPNQ0095 (250 mg)

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.

HANDLING

PACKAGING AND STORAGE

Store beads at 2–8°C.

EXPIRATION

Once Reconstituted, the beads are stable for up to 7 days when stored in the appropriate conditions.

SAFETY WARNINGS AND PRECAUTIONS

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 statement(s) for specific advice.

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.

COMPONENTS OF THE ASSAY SYSTEM

This pack contains the following assay component, sufficient material for at least 500 tests.

SPA bead
Polyvinyltolune (PVT) copper his-tag SPA beads, as a suspension in water at 20 mg/ml.

DESCRIPTION

The PVT copper his-tag SPA beads is a novel bead formulation designed to use the scintillation proximity assay (SPA) principle. In a direct assay format, the SPA beads could be used to trap and quantify the binding of a directly-radiolabelled histidine (his)-tagged fusion protein, peptide or oligopeptide (such as a kinase substrate, using [^{33}P]ATP as the donor molecule). In an indirect assay format, the SPA beads could be used to trap and quantify the association of a radiolabelled binding partner to a histidine (his)-tagged fusion protein, peptide or oligopeptide.

The SPA system is based on a scintillating PVT bead. The outer surface of the bead has been modified by a coating of a chemical chelate (containing bound copper) which enables the binding of histidine-tagged fusion moieties.

Evaluation studies have been performed using the direct binding to the SPA bead of a model [^{3}H]tyrosine-(histidine)$_{6}$-alanine ([^{3}H]YHHHHHHA) peptide. Only the peptide bound via the metal-chelate coating to the bead will generate a significant signal. Unbound peptide in the supernatant will not be in close enough proximity to generate a light signal.

The SPA protocol is very simple to perform, and compared to traditional methodology has many advantages:

- No filtration or washing steps to separate bound from free moieties are required
- Only pipetting steps are necessary
- Use of liquid scintillant is not required
- The system is amenable to automation.

Assay precision is high and substantial time saving can be achieved over existing methodology. The assay could easily be used in a high throughput format .

CRITICAL PARAMETERS

The following points are critical:

- The assays are performed in phosphate-buffered saline (PBS) containing 0.5% (final concentration) bovine serum albumin (BSA). This is performed by diluting the beads to a 2 × concentration in water (pH 7.0), and then further diluting the beads (2-fold) into 2 × PBS containing 1% BSA. 2 × PBS is made by taking 1 PBS tablet. (Sigma, Product code P-4417) and dissolving into 100 ml of water at pH 7.0. Changes in pH may affect the binding of his-tagged protein to the bead.
- All studies have used [^{3}H] as the radiolabel. Alternative labels have not been evaluated although it is speculated that the use of [^{125}I] and [^{33}P] would be feasible. Studies with [^{33}P] in kinase assays are currently under investigation. See section on additional information on the use of [^{33}P] with SPA.

- Suitable controls need to be set up. For example, if the assay type is for the trapping and quantifying of his-tagged protein-DNA or his-tagged protein-protein interactions, then the ideal control would be to omit the his-tagged protein. Addition of imidazole (0.1 M final concentration) should inhibit the large majority of copper-chelate-dependent binding.

- The quantities of bead and other reagents such as peptides, binding partners and enzymes, need to be optimised by the researcher.

- When using highly coloured samples, colour quench correction may be necessary.

ADDITIONAL EQUIPMENT AND REAGENTS REQUIRED

The following equipment and reagents are required but are not supplied:

- Assay buffer (2 × concentrate PBS containing 1% BSA, pH 7.0). For 100 ml, add 1 PBS tablet (Sigma, Code P-4417) to 100 ml water, and dissolve 1 g BSA into this. The pH should be adjusted to 7.0 using appropriate acids or bases.

Direct assay format

- His-tagged fusion moiety (such as a peptide, protein or oligopeptide in a suitable concentration for the assay) and appropriate radiolabel donor and enzyme, if needed.

Indirect assay format

- His-tagged fusion moiety and radiolabelled binding partners.
- Microplate scintillation counter.
- 96-well microplates compatible with the microplate scintillation counter.
- Self-adhesive microplate seals.
- Ice bath for the temporary storage of all reagents.
- Pipetting equipment, either manual or automated systems (for example, 10 µl, 50 µl, 100 µl, 1 ml).

Microplate scintillation counters and plate sealers are available from PerkinElmer.

ASSAY PROCEDURE

(For the trapping and quantifying of his-tagged proteins and their binding partners)

It is proposed that the following protocol or similar variations would be suitable for quantifying radiolabelled binding partners to his-tagged moieties using the SPA beads, or the direct quantification of radiolabelled his-tagged species.

REAGENT PREPARATION

Bead in assay buffer

1. PVT copper his-tag SPA beads are supplied as a suspension in water, containing 250 mg total per vial, at a concentration of 20 mg/ml. This material should be stored protected from light at 2–8°C. **The SPA beads should be mixed to ensure a homogeneous suspension while pipetting.** This may be done by continuous agitation with a magnetic stirrer or vortex mixing. **Important note: magnetic stirrers bars, if employed, should be completely clean, coated with a chemically-inert material, and free from any surface-bound metals or metal salts.** Beads should be diluted to 2 × required working concentration in water, and then diluted 2-fold into the 2 × PBS/1%BSA buffer (see example below). Keep the beads in this buffer at 2–8°C for the duration of the assay.

2. For **one** 96-well plate using 500 g (bead per well, remove 50 mg (2.5 ml) of SPA bead as supplied (20 mg/ml) **into a clean glass container.** Add water (2.5 ml) to the bead suspension and vortex mix. This produces a bead stock at 10 mg/ml. Dilute this new bead stock at 10 mg/ml (5 ml) with 2 × PBS containing 1%BSA (5 ml). 100 µl of this working bead stock now in PBS/0.5%BSA, will contain 500 µg bead. Store on ice.

Please note: that a small excess volume of bead in assay buffer will be generated to allow for pipette variation.

ASSAY PROTOCOL

(for a final assay volume of 120 µl–130 µl. This volume could of course be reduced, if appropriate)

1. Prepare reagents as described in the previous section.

2. Label plates/wells as required.

For an indirect format assay, incubate the required concentration of his-tagged protein (at a suitable temperature and for an optimized time) with its radiolabelled binding partner in an appropriate buffer in the microplate wells. A suitable inhibitor or competitor of the binding interaction may be added here if required. The volume at this stage should be no more than 20 µl. **For a direct format assay** (such as a kinase assay, where the his-tagged protein acts as a substrate), incubate the required concentration of his-tagged protein (at a suitable temperature and for an optimized time) with a quantity of reagents such as an enzyme and radiolabel donor, in an appropriate buffer in the microplate wells. A suitable inhibitor or competitor of the binding interaction may be added here if required. The volume at this stage should be no more than 20 µl.

3. Suitable controls should be set up. For example, the incubation of the radiolabelled binding partner with the SPA bead in the absence of the his-tagged-tagged protein, omission of an enzyme or addition of histidine (e.g. 0.1 M final concentration).

4. Add 100 µl of his-tag SPA bead (0.5 mg) to each well.

5. Seal plate with appropriate stickers.

6. Allow plates to incubate at room temperature for 60 minutes. Shaking may be needed.

7. Count each well for 1 minute in a β-scintillation counter.

ADDITIONAL INFORMATION

REAGENT COMPATIBILITY

The assay may be conducted within the pH range 6.0–8.0. Table 1 below indicates the effect on SPA counts by the addition of a range of reagents.

Table 1. The effect of a range of assay reagents.

Reagent	Final assay concentration	% of control signal
Ethanol	5%	96
Methanol	5%	98
DMSO	5%	94
SDS	5%	85
Chaps	5%	94
Triton X-100	2.5%	97
$MgCl_2$	20 mM	97
$CaCl_2$	20 mM	97
NaCl	150 mM	97
KCl	150 mM	96
EDTA	125 µM	90
Imidazole	8 mM	90
Histidine	500 µM	90
Hepes	50 mM	100
MOPS	50 mM	100
TRIS	50 mM	100
ATP	50 mM	98
DTT	1 mM	1

It remains the responsibility of the user to determine whether assay reagents are interfering with the reaction by the inclusion of appropriate controls.

USE OF [^{33}P] WITH SPA

When working with [^{33}P] , the SPA beads are allowed to settle or are centrifuged prior to counting. This penultimate step is important due to the relatively high maximum β-energy (0.249 MeV) of the decaying [^{33}P] isotope and the concomitantly high mean path length (0.6 mm) of the β-particle. What this means for SPA is that, were the free [^{33}P] counted with the beads in co-suspension, the non-specific proximity effects (excitation of bead fluor by unbound isotope) would be substantial. This can be overcome either by allowing the beads to settle out under gravity or by pelleting the beads using a centrifuge.

BACKGROUND AND REFERENCES

Co-ordination of metals to solid surfaces for the binding and exchange of small molecules leading to chromatographic separation has been known since the 1960's (1). However, it was Porath in 1975 who introduced the term Immobilised Metal Affinity Chromatography (IMAC) for the separation of proteins (2). Iminodiacetic acid (IDA)

and TED (tris(carboxymethyl)ethylene diamine) were the original polymer-bound chelators employed by Porath, which bind a range of first-row transition metals such as Ni^{2+}, Zn^{2+}, Fe^{3+} and Cu^{2+}. Further chelators based on EDTA (ethylenediamine tetraacetic acid) and similar structures have also been described in the literature (3). The chelators bind metal ions via nitrogen atoms and carboxylate residues, with remaining sites occupied by water molecules or buffer ligands. Upon addition of moieties containing notably histidyl residues, competition occurs, displacing the water molecules or buffer ligands with the histidine, via the latters' ε- or δ-nitrogen.

The property of these metal-chelate complexes for use in affinity chromatography has been extended to purification of histidine-tagged fusion proteins and oligopeptide hybrid fusion products.

Using this concept for immobilising his-tag fusion proteins, the copper his-tag SPA bead has been developed. This bead will enable the trapping and quantification of histidine-tagged-fusion proteins and their binding partners using SPA technology.

The ability to trap and quantify cloned tagged proteins is a desirable target in the drug screening market, potentially replacing techniques such as yeast hybridization systems (4), immunoprecipitation, electrophoresis and blotting.

In many cellular processes, protein-protein interactions are of major importance. Studies of the kinetics of these interactions have been simplified by the direct measurement of their binding using SPA beads. The immobilisation of histidine-tagged fusion proteins onto SPA beads and the direct measurement of interaction with their binding partners has a number of potential advantages. It reduces the number of manipulations in the assay because the link between the bead and the fusion protein is simplified. In addition, modification of the fusion protein itself is not required.

1. DAVANKOV, V.A. and SEMECHKIN, A.V., *J. Chromatog.,* 141, pp.313–353, 1977.

2. PORATH, J. *et al., Nature,* 258, pp.89–599, 1975.

3. HANER, M. *et al., Anal. Biochem.,* 138, pp.229–234, 1984.

4. FIELDS, S. and SONG, O-K., *Nature,* 340, pp.245–246, 1989.

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