YSi (2-5 µm) Copper His-Tag SPA Beads

Product Number: RPNQ0096

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.

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.

Description

The YSi (2–5 µm) 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-radiolabeled histidine (his)-tagged fusion protein, peptide or oligopeptide (such as a kinase substrate, using [33P]ATP as the donor molecule). In an indirect assay format, the SPA beads could be used to trap and quantify the association of a radiolabeled binding partner to a histidine (his)-tagged fusion protein, peptide or oligopeptide. The SPA system is based on a scintillating yttrium silicate particle (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 [3H]tyrosine-(histidine)6-alanine ([3H]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.

Critical Parameters

The following points are critical:

▪The assays are performed in phosphate-buffered saline (PBS) containing 0.2% (final concentration) bovine serum albumin (BSA). This is performed by diluting the beads to a 2 x concentration in water (pH 7.0), and then further diluting the beads (2-fold) into 2 x PBS containing 0.4% BSA.   Changes in pH may affect the binding of histagged protein to the bead.
▪ All studies have used [3H] as the radiolabel.
▪ 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 optimized by the researcher.
▪ When using highly colored samples, color quench correction may be necessary.

BEAD RECONSTITUTION

Bead Preparation

. YSi (2–5 μm) copper his-tag SPA beads are supplied as a suspension in water, containing 125 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 chemicallyinert
material, and free from any surface-bound metals or metal salts. Beads should be diluted to 2 x required working concentration in AnalaR grade water, and then diluted 2-fold into the 2 x PBS/0.4% BSA buffer. Keep the beads in this buffer at 2–8°C for the duration of the assay.
2. For one 96-well plate using 250 μg bead per well, remove 25 mg (1.25 ml) of SPA bead as supplied (20 mg/ml) into a clean glass container. Add AnalaR grade water (3.75 ml) to the bead suspension and vortex mix. This produces a bead stock at 5 mg/ml. Dilute this new bead stock at 5 mg/ml (5 ml) with 2 x PBS containing 0.4% BSA (5 ml). 100 μl of this working bead stock now in PBS/0.2% BSA, will contain 250 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.

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