nanoSPA Scintillation Proximity Assay Nanoparticles: Determining the Amount of nanoSPA for Your Assay

For Research Use Only

Storage

nanoSPA™ is shipped in 10 mM HEPES pH 7.4 at 10 mg/mL. Store nanoSPA™ in the refrigerator (4 °C) or at room temperature (approximately 25 °C).

Description

nanoSPA™ is a proprietary nanoparticle scintillator that presents several advantages over traditional liquid scintillation cocktails and solid scintillation materials. nanoSPA™ is small, has an intermediate density compared to polymer or inorganic crystal scintillators, and is readily dispersed in water compared to inorganic particles which settle and organic particles which can aggregate. The particles are functionalized at the surface with chelating groups and have already been loaded with Ni^{2+} for use with his-tagged proteins. His-tagged proteins can be attached to the nanoparticle surface by combining the nanoparticles with the his-tagged protein in buffer and mixing gently on a shaker or similar device for 2 hours, or by using protocols similar to those used for attaching protein to Ni-NTA purification beads. Beads may or may not be rinsed to removed unattached protein as desired.

Summary

nanoSPA™ is a nanoparticle scintillator for scintillation proximity assays (SPA) in aqueous samples. Scintillation proximity assay (SPA) is a derivative of solid scintillation counting, wherein specific binding of radiolabeled analyte to a solid scintillator surface increases the probability of energy absorption by the scintillant. SPA is particularly useful for β-emitters with low penetration depths and results in an increased number of emitted photons upon analyte binding. SPA also eliminates the need to separate bound from unbound analytes in radioimmunoassays, markedly enhancing the throughput and simplicity of the assay. Consequently, SPA lends itself to the monitoring of binding kinetics under steady state conditions, as well as to quantification of radiolabeled analytes using automation and high-throughput screening methods.

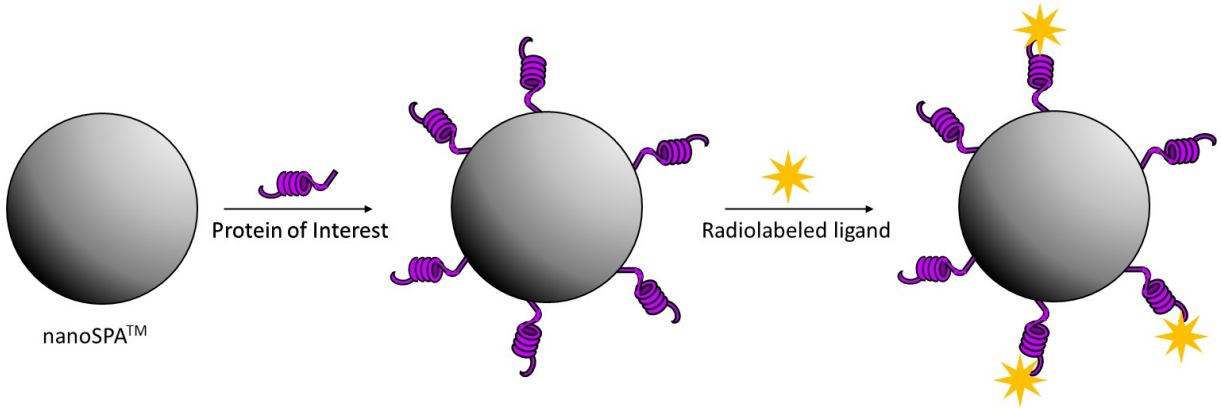

Figure 1. Illustration of a nanoSPA™ particle functionalized to bind a protein of interest, which can be used to study the binding of the protein to a radiolabeled ligand. Signal is very low before ligand binding, but after the ligand is bound to nanoSPA by surface-attached protein of interest, the probability that energy from radiolabeled decay will be absorbed by the nanoSPA increases due to proximity. Consequently, the signal increases.

Materials and Equipment

Assay Buffer

Scintillation vials (plastic or glass), or 96-well plate

Scintillation Counter (for scintillation vials or for microplates)

Pipettes and pipette tips

His-tagged (6× or 10×) protein of interest

Radiolabeled ligand for protein of interest (^{3}H, ^{35}S, ^{33}P or ^{14}C labeled)

Safety

Please follow all safety protocols for storage, use, and disposal of radioactive materials approved by your institution.

nanoSPA™ Concentration Test Protocol

It is recommended that you initially try several concentrations of nanoSPA to determine the concentration that provides the greatest signal-to-noise ratio for your assay. A general protocol is provided below.

1. nanoSPA™ is shipped in 10 mM HEPES pH 7.4 at 10 mg/mL. Remove the amount needed for your test assay according to Table 1 below.

   Table 1: Amounts and volumes of nanoSPA™ needed for concentration testing.

Type	20 mL Vial	7 mL Vial	96-well Plate
Sample size	10 mL	5 mL	200 µL
Amount of nanoSPA™	135 mg	67.5 mg	2.7 mg
Volume of nanoSPA™ stock	13.5 mL	6.75 mL	270 µL

2. Centrifuge the nanoSPA™ slurry at approximately 10,000 × g, then disperse the particles in the chosen assay buffer at 10 mg/mL.
3. Prepare samples by adding the volumes of nanoSPA™ in assay buffer to scintillation vials or plate wells as shown in Table 2.
4. Add the his-tagged protein of interest at the desired concentration (e.g. the concentration you plan to use for your future experimental assays) to each sample vial or well. The theoretical maximum for loading is 50 nanomoles per mg of nanoSPA™. It is not recommended that you exceed this theoretical maximum, as you may waste some of your his-tagged protein of interest. Ensure that the concentration of your protein is high enough that the total volume for each sample is not exceeded. Additional assay buffer should be added, if necessary, to reach the total volume for the format chosen (20 mL vial, 7 mL vial, or 96-well plate).
5. Allow your his-tagged protein of interest to bind to the nanoSPA™ at temperatures between 4 and 37 °C (depending on your protein) for at least 2 hours.
6. Prepare samples for background measurements by adding the volumes of nanoSPA™ in assay buffer to scintillation vials or plate wells as shown in Table 2, omitting his-tagged protein. Add assay buffer to the volume of the chosen format.

Table 2: Sample volumes for individual wells. nanoSPA™ is 10 mg/mL in assay buffer, unless otherwise noted. The same concentration of his-tagged protein is added to all samples.

Test nanoSPA™ Concentration	20 mL Vial (10 mL total volume)	7 mL Vial (5 mL total volume)	96-well Plate (200 µL total volume)
3.0 mg/mL	3 mL nanoSPA™	1.5 nanoSPA™	60 µL nanoSPA™
2.0 mg/mL	2 mL nanoSPA™	1 mL nanoSPA™	30 µL nanoSPA™
1.0 mg/mL	1 mL nanoSPA™	0.5 mL nanoSPA™	15 µL nanoSPA™
0.5 mg/mL	0.5 mL nanoSPA™	0.25 nanoSPA™	30 µL 2.5 mg/mL nanoSPA™
0.25 mg/mL	0.25 mL nanoSPA™	0.125 nanoSPA™	15 µL 2.5 mg/mL nanoSPA™

7. Add the same amount of radiolabeled ligand to all 10 samples. Allow the radioligand to bind to your his-tagged protein for the same time and at the same temperature (between 4 and 37 °C) that you plan to use for your experimental assay.
8. Read sample vials or wells in your scintillation counter.
9. Compare the count per minute (CPM) or disintegrations per minute (DPM) of samples of each concentration of nanoSPA™ with the background for each concentration of nanoSPA™.

$$\frac{\text{CPM or DPM of sample with his-tagged protein of interest}}{\text{CPM or DPM of sample without his-tagged protein of interest}} = \text{Signal to Background Ratio}$$

The nanoSPA™ concentration that exhibits the highest Signal to Background Ratio should provide you with the best results for your experimental assay.