For Research Use Only Not for Diagnostic Use

Express Capture Nickel Coated Plates

A rapid screening, enzyme assay, and protein purification tool from XpressBio.

Catalog Numbers:

EX9000-C96 (Clear 96 well plate)
EX9000-B96 (Black 96 well plate)
EX9000-W96 (White 96 well plate)
EX9000-C384 (Clear 384 well plate)
EX9000-B384 (Black 384 well plate)
EX9000-W384 (White 384 well plate)

Product Description

Express Capture Nickel Plates provide a platform for the capture, enzymatic assay, and purification of recombinant histidine-tagged proteins that is reliable, fast, and has high throughput capabilities. Six consecutive histidine residues form an electron cloud with a net negative charge that can bind, through a salt linkage, to positive charged metal ions such as nickel. This binding activity of His-6 and Ni2+ is utilized when recombinant cDNA is expressed into protein using several commercially available expression vectors. The His-6 tagged recombinant protein can be dissociated from the nickel ion with the competitive inhibitor of histidine, imidazole. This product may be used with His-tagged proteins or peptides for various uses such as recombinant protein expression screening, immunoabsorbtion assays, biochemical assays, competition assays and protein purification. As little as 50 pg of His-tagged protein can be detected in a well.

Components

One nickel coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant/humidity indicator card. The plate should be stored at refrigerated temperature (4 °C). Unused strips should be stored resealed in the foil pouch. The product is stable for one year when stored properly.

Materials and Equipment Required But Not Provided

His-tagged proteins
Detection enzyme-conjugate
Enzyme substrate
Pipettes and Tips (Multi-channel)
Wash bottle or automatic plate washer
Microtiter plate reader

Procedures

I. Coupling of His-tagged Proteins

Buffers:
PBS; Phosphate Buffered Saline, pH 7.4, 10 mM Sodium Phosphate, 2 mM Potassium Phosphate, 135 mM Sodium Chloride, 2.7 mM Potassium Chloride.
Imidazole; 5 to 400 mM in PBS
Blocking Buffers: 1. Dissolve casein (purified powder from Sigma C-5890) to 2 gm/L (0.2%) in PBS-T. Heat to 37° C and stir until dissolved; or 2. Dissolve probumin (Millipore, Catalog# 82-045-1) or BSA to 5 gm/L (0.5%) in PBS-T. Heat to 37° C and stir until the albumin is dissolved.

Recommended Concentrations:

His-tagged proteins: 50 pg to 3 ug diluted in PBS supplemented with 5-10 mM imidazole

Protocol:
1. Using a wash bottle or automated washing equipment add 250-300 µl of PBS buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure two times (for a total of three washes).
2. Prepare the his-tagged protein in PBS supplemented with 5-10 mM imidazole.
3. Add 100 µl of the protein solution to the microtiter plate.
4. Incubate for one hour at room temperature. Agitation on a rotary mixer can be used (100 rpm).
5. Remove the liquid and wash using the wash protocol in step one with PBS buffer, repeat this wash step 3 times.

6. Optional Blocking Step. In applications were the bound protein would be assayed directly in the plate, a method to reduce assay background

is to include a blocking step after washing away the GST-tagged protein sample with PBS-T. Add 100 µl of a blocking buffer to each well and incubate for 45 minutes at room temperature. Aspirate the wells and wash five times with PBS-T, blotting between washes.

II. Detection of His-tagged Protein

Many methods can be utilized to detect the captured His-tagged proteins. It will usually be an antibody specific for the protein of interest, followed by a secondary antibody conjugated to an enzyme, such as peroxidase, that will attach to the primary antibody. The enzymatic activity gives a time dependent increase in color at a specific wavelength of light that can be detected with a microplate reader. However, direct biochemical assays specific for the protein or enzyme of interest can also be used. The following example illustrates the detection of recombinant rat NS1 protein with rat anti-NS1 anti-serum followed by a secondary goat anti-rat anti-body conjugated with peroxidase. The relative activity of bound His-tagged protein is quantitative using ABTS peroxidase substrate.

1. Make an appropriate dilution (1/5 to1/2000) of the primary antibody, rat anti-NS1 antibody, in PBS. Add 100 µl of each dilution to a microtiter plate well that has been coupled with the His-tagged NS1 protein (see above).
2. Incubate at room temperature for 60 minutes.
3. Aspirate the wells and wash three times with PBS buffer as described above.
4. Add 100 µl of goat anti-rat antibody conjugated with peroxidase and incubate at room temperature for 45 minutes.
5. Aspirate the wells and wash three times with PBS buffer as described above.
6. Add 100 µl of ABTS peroxidase substrate solution and incubate at room temperature for 10 to 20 minutes. Read the plate on a microplate reader set to 405 nM.

III. Purification of His-tagged Protein

The His-tagged protein can be eluted from the nickel coated plate with imidazole in PBS. To purify the His-tagged protein the wells can be washed with low concentrations of imidazole.
1. Add 100 µl of imidazole in PBS at concentrations ranging from 10 to 30 mM.

2. Incubate at room temperature for 2 minutes and aspirate the wells. Repeat 2 to 5 times.

3. Elute the His-tagged protein from the nickel coated plate by adding 120 µl of 400 mM imidazole in PBS and incubating at room temperature for 5 minutes. Draw off the protein solution and place in a clean microcentrifuge tube for further analysis.

Critical Steps

1. Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 ul) is recommended. Complete removal of the wash buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.
2. High backgrounds can occur from inadequate washing or from too highly concentrated detection materials. Careful titration of ligands will result in optimal assay.
3. Low signals can also occur from inadequate titration of detection materials.
4. All reagents must be at room temperature before running the assay. Remove only the volume of reagents that is needed. Do not pour reagents back into vials as reagent contamination may occur.
5. The optimal imidazole concentration to wash and elute a specific His-tagged protein from the nickel coated plate depends upon the protein of interest and should be determined experimentally.

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