

MSD Multi-Array™ Polyhistidine Binding Plates

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1 Abstract

Proteins are often expressed in bacteria as fusion proteins containing a fusion tag. The fusion tag has a strong affinity for a known ligand and is exploited to purify the protein. Polyhistidine or His-tag, a stretch of 3 to 6 consecutive histidines, is a commonly used fusion tag with a strong affinity for divalent metals.

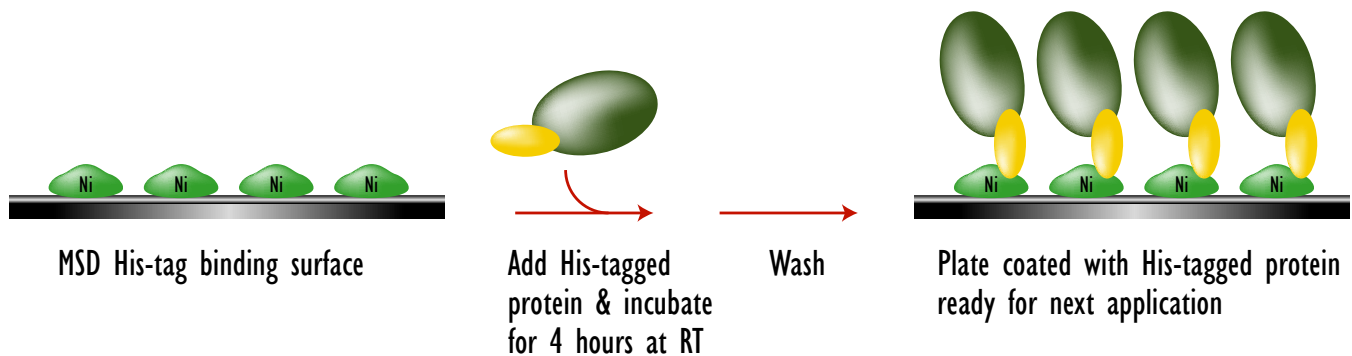
Meso Scale Discovery has developed Multi-Array™ plates that can capture His-tagged proteins. We demonstrate the use of these plates and MSD's SECTOR™ Imager 6000 to carry out highly sensitive assays that can detect as little as 0.1 fmol of a His-tagged protein. The plates have a high binding capacity for His-tag proteins (> 1 pmole per well), and exhibit **low non-specific binding**.

We also demonstrate the use of these plates in a functional assay measuring the ubiquitinylation of a His-tagged E2 ubiquitin ligase.

2 Protocol

MSD His-tag binding plates are offered in 96-well and 384-well formats.

The His-tagged species is incubated in MSD diluent buffer for 4 hours. Plates are washed. The Ni^{2+} -presenting surface is now coated with the His-tagged protein and ready to be used in the next step.

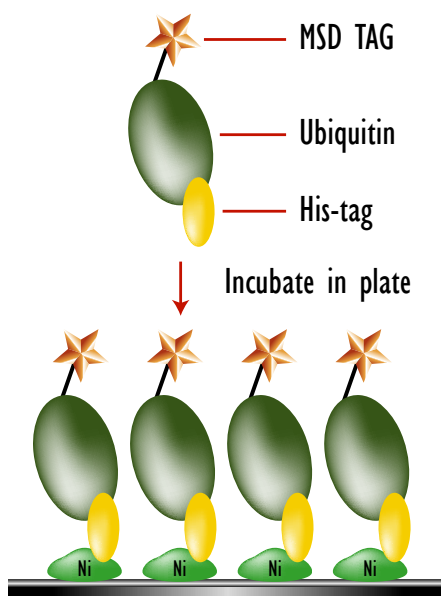


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3 Sensitivity and Binding Capacity

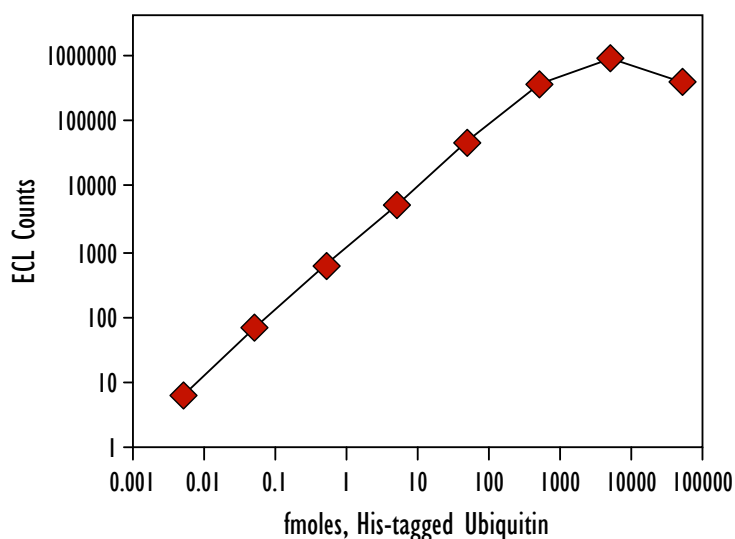
His-tagged ubiquitin (MW 10kD), which was chemically modified with the electrochemiluminescent label MSD TAG™ was used to characterize MSD His-tag binding plates.



His-Tagged Ubiquitin on MSD 96-well His-Tag Binding Plates

Detection Limit is 0.044 fmoles

Binding Capacity is 1250 fmoles



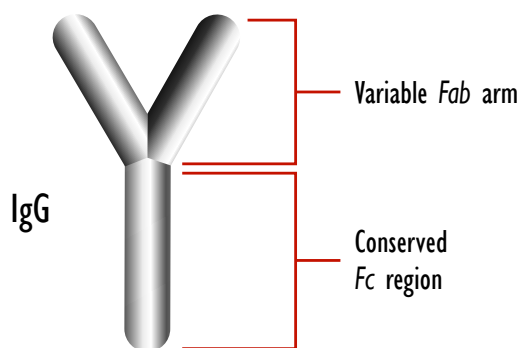
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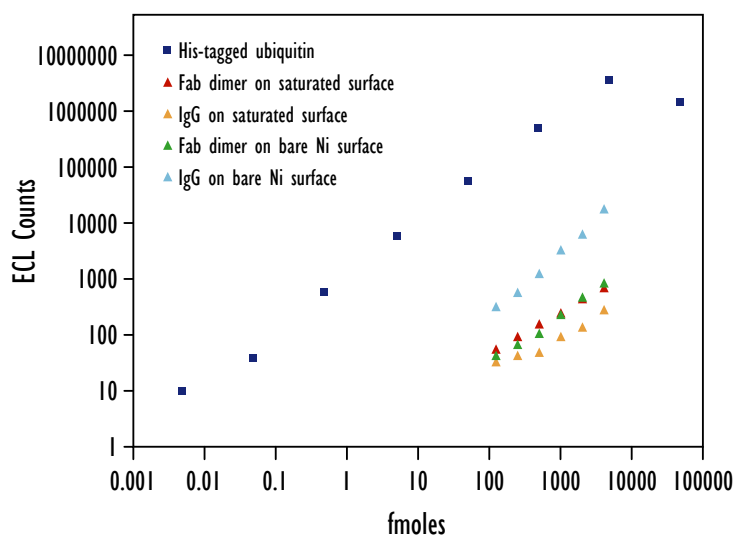
4 Non-Specific Binding

IgG species are known to contain a stretch of histidine residues in their Fc region. Consequently, they are expected to give rise to non-specific binding on polyhistidine binding plates. This is not a serious issue if the surface is saturated with a His-tagged protein. However, for under-saturated polyhistidine binding surfaces, use of Fab fragments is recommended.

MSD formulated diluents in which His-tagged species can be incubated decrease non-specific binding exhibited by IgG species significantly.

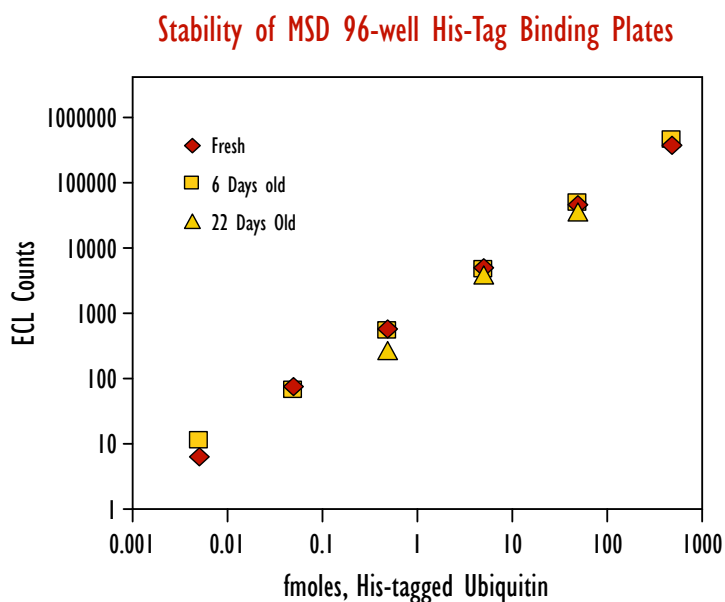


Specific and Non-Specific Binding on MSD 96-well His-Tag Binding Plates



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5 Stability



Plates were stored at 4°C.

There is no detectable increase in non-specific binding as the plates age.

6 MSD His-Tag Plates Presenting Different Metal Surfaces

His-tag binding plates can be modified to be coated with custom metal surfaces for specific applications

Divalent Metal	ECL Counts
Ni ²⁺	211,083
Zn ²⁺	253,790
Fe ²⁺	103,841
Cu ²⁺	181,196

Histidine-tagged ubiquitin was used as the analyte.

Different divalent metals yield slightly different signal levels.

There are no significant differences between non-specific to specific signal ratios observed on different metal surfaces.



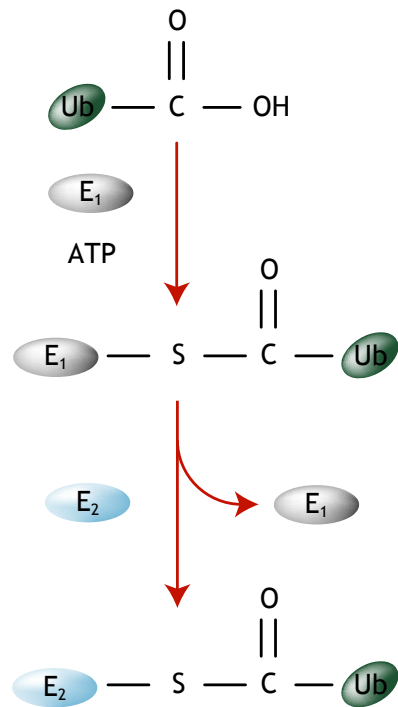
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7 Use of MSD Polyhistidine Binding Plates in an Assay Probing the E2-Ubiquitin Interaction

Ubiquitylation involves transfer of ubiquitin through sequential action of ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2) and ubiquitin ligase enzyme (E3).

This assay tests for the presence of the E2-Ub interaction.



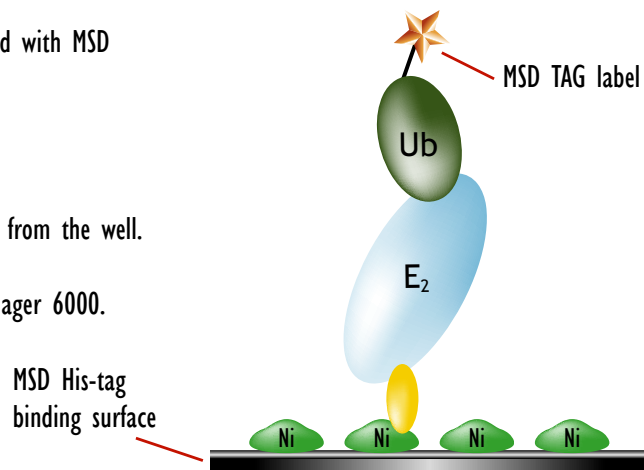
8 Assay Format

A reaction mixture of E1, His₆-E2, ATP, Mg²⁺, and ubiquitin labeled with MSD TAG (Ub*) is incubated.

The E2-Ub* conjugate is captured through His₆-Ni²⁺ interaction.

Excess Ub* and other upstream reaction components are washed from the well.

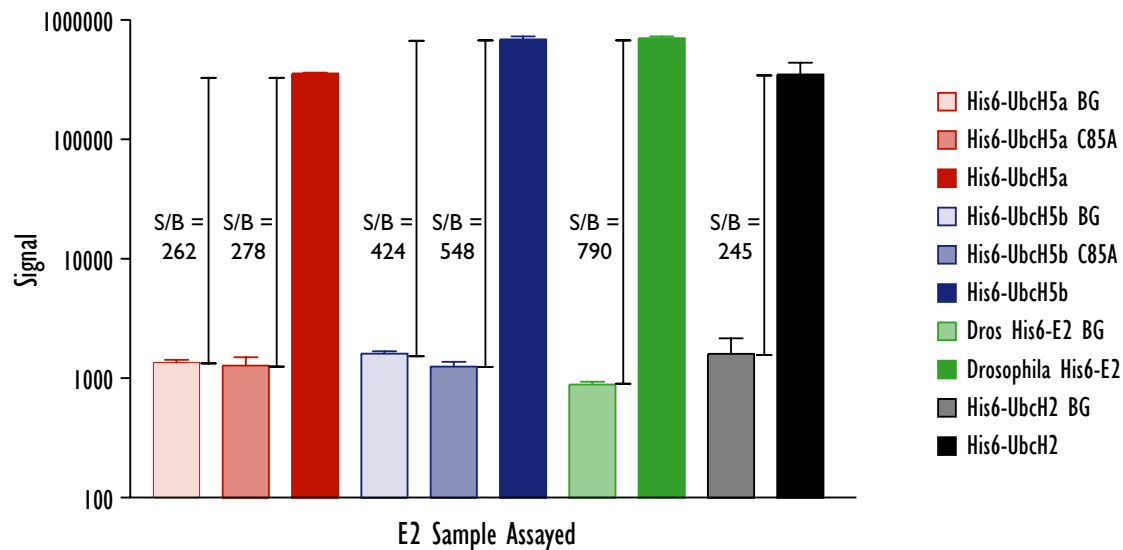
The plate is read with MSD Read Buffer on the MSD's SECTOR Imager 6000.



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9 Assay Validation: Signals generated by selected E2s



For all E2 samples, background (BG) signals are defined as assays excluding E1.

Mutant UbcH5a (C85A) and UbcH5b (C85A) both generate comparable signals to the background.

10 Conclusions

MSD His-tag binding plates have high sensitivity (< 0.1 fmole), practical binding capacity (1 pmole) and very low non-specific binding.

MSD His-tag plates exhibit very comparable sensitivity to the best technology available currently in the market, but have the added advantage of being offered in multiple formats. The current repertoire of 96-well and 384-well His-tag binding plates will soon be expanded to include the patented Multi-Spot™ plate formats.

MSD plates can be coated with a divalent metal of choice for specific applications.

MSD diluents for the incubation of His-tagged species in MSD His-tag binding plates suppress non-specific binding efficiently and MSD plates exhibit much lower non-specific binding than the current technologies in the market.



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