

Introduction

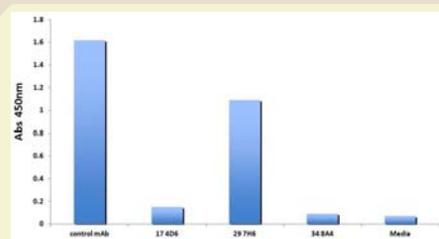
ProSci Inc. is a provider of Custom Antibody Services and also maintains an extensive broad-based catalog of reagent antibodies. Our company has provided over 12,000 Custom Antibody Projects that have generated polyclonal, monoclonal and single-domain antibodies. Here we describe antibodies generated against the human immune regulator protein PD-1 and some of the properties and applications of these antibodies.

Immunogen: extracellular domain of human PD-1 expressed and purified from HEK293 cells.

Antibody types: murine monoclonal antibodies (mAb) and llama single domain antibodies (sdAbs).

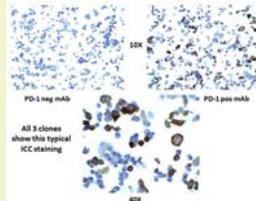
Antibodies were initially screened for binding to the extracellular domain of human PD-1 via ELISA. Subsequent screening was performed to test mAb applications in various assays including: Western, live-cell binding, ICC, peptide mapping, and inhibition of ligand (PD-L1) binding.

Initial data from llama sdAbs is also presented.



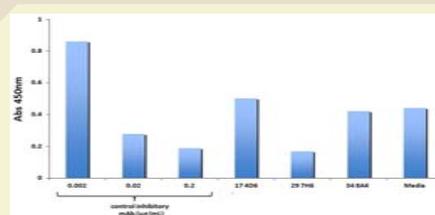
Live-cell ELISA

Various ELISA positive mAbs were screened for their ability to bind PD-1 expressed on the surface of live cells. PD-1 transfected HEK293 cells were grown in microtiter wells and 48 hrs post-transfection incubated with various mAbs. Antibodies were detected with anti-IgG-HRP conjugates. Mock transfected cells were not recognized by any mAbs (data not shown). Both an anti-PD-1 control mAb and mAb #29 bind to live cells expressing PD-1.



ICC Staining

All mAb clones were tested for their ability to stain transfected HEK293 cells by ICC methods. Three clones #17, #29, and #34 showed positive staining for PD-1.



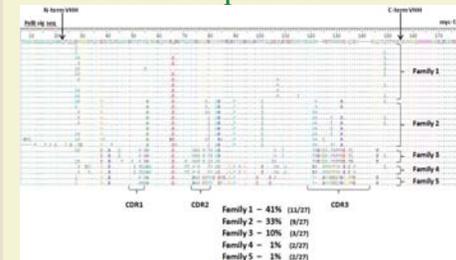
Inhibition of PD-L1 Binding

mAbs supernatants were tested for their ability to block the binding of PD-L1 to PD-1. PD-1 was coated on ELISA plates followed by incubation with a control mAb or hybridoma supernatants. Biotinylated PD-L1 was added and bound PD-L1 was detected by streptavidin-HRP. mAb#29 shows noticeable inhibition of PD-L1 binding to PD-1.

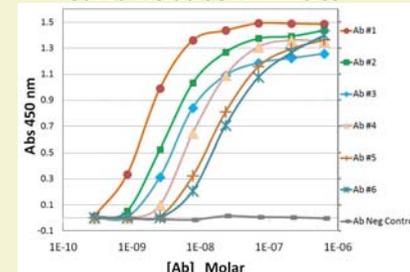
Summary of PD-1 mAb Applications

| Antibody | Ag ELISA | Western | Live cell Binding | ICC | Peptide binding | Inhibition of Ligand Binding |
|----------|----------|---------|-------------------|------|-----------------|------------------------------|
| 17 4D6 | ++++ | +++ | - | ++ | +++ | - |
| 29 7H6 | ++++ | - | +++ | +++ | - | ++ |
| 34 8A4 | ++++ | ++++ | - | ++++ | - | - |

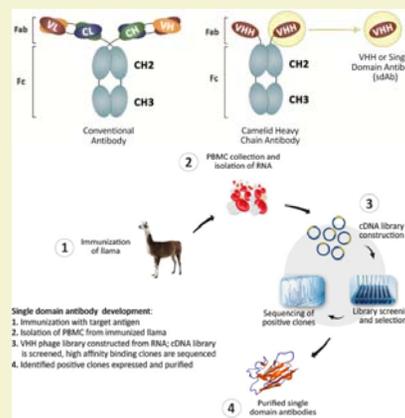
PD-1 sdAb Sequence Families



sdAb Relative Affinities



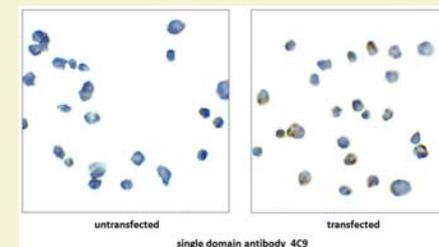
ProSci Single Domain Antibodies



PD-1 Single Domain Antibodies

We have recently used ProSci's Single Domain Antibody development capabilities to generate sdAbs against PD-1.

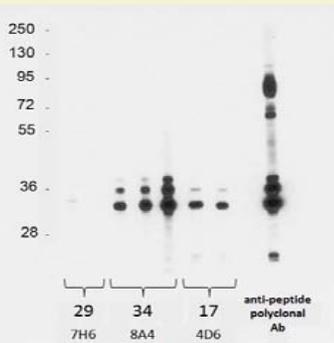
ICC of PD-1 Transfected HEK293



Summary

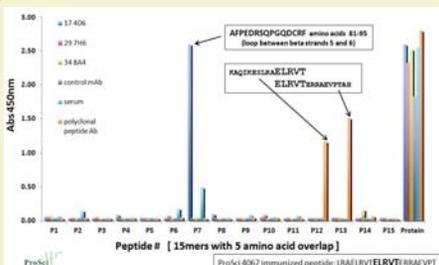
We have generated murine mAbs against the extracellular domain of human PD-1 and identified application specific and functional mAbs. Further analyses of these mAbs and additional screening are continuing. Single domain antibodies against PD-1 were also generated with some initial characterization.

ProSci's custom antibody services are available for supporting your needs in antibody drug discovery by developing application specific and functional antibodies.



Western analysis of mAbs directed against PD-1

HEK293 cells were transfected with an expression plasmid containing the full length human PD-1 gene. 48 hrs post-transfection cell lysates were prepared and analyzed by Western blot. Samples were probed with various mAbs initially screened for antigen recognition via ELISA. mAbs #34 and #17 strongly recognize what are likely various glycosylated forms of PD-1.



Peptide Epitope Mapping

PD-1 mAbs were tested by a peptide scanning strategy. 15mer peptides were synthesized across the sequence of the extracellular domain of PD-1 starting at Pro21. Each peptide has a 5 amino acid overlap with the adjacent peptide. mAbs were tested for binding via ELISA. One mAb, #17, recognizes an epitope in peptide P7 which appears to be in a loop between beta strands 5 and 6. As a positive control, peptides P12 and P13 are recognized by a ProSci PD-1 anti-peptide antibody (4067).