Applied StemCell, Inc.
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Analysis Report

Monoclonal Antibody Development
Stage 1-2 Report for Project #XXXXX

Shipping Materials:

Catalog number CXXXX-X Antibody plates/ Serum sets for evaluation QTY: 1 set

The material is for strictly laboratory academic research purposes only in academic laboratory. Item is not used for any in vivo use.
The material does not include any hazardous or dangerous materials. Material is not infectious.
The material does not include any IATA-regulated materials for transportation.

Shipping temperature: Dry Ice or Blue Ice

Project Description:

The client requested to develop rabbit monoclonal antibodies against 7Aa and 9Aa peptides of a protein. The client synthesized the two peptides, and a cysteine was added to either the N-terminus of the peptide to facilitate protein conjugation. The sequences of the peptides are:

- 7Aa: XXXXXXX
- 9Aa: XXXXXXX

Peptides were successfully synthesized and passed purity and solubility test. They were conjugated to Keyhole limpet hemocyanin (KLH) for rabbit immunization. They were also conjugated to bovine serum albumin (BSA) to be used in testing. Two rabbits (XXX and XXX) were immunized with a mixture of the 2 KLH conjugated peptides following our standard immunization protocol. Serum samples were collected before (Prebleed), or after 3 immunization injections (Postbleed 1), or after 4 immunization injections (Postbleed 2). A 96-well ELISA plate was coated with each of the two BSA conjugated peptides. Experiments were carried out with a 5-fold serial diluted serum samples starting from 1:200.

Stage 1. Result and Summary

The serum titer ELISA results are as below.

Summary: for all 2 peptides, the serum titer exceeded 1:625,000 after 4 injections, indicating successful immunization. Proceeding to Stage 2 is recommended.

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Coated protein	XXX-XXx					
Animal	XXX			XXX		
Bleed	Prebleed	Postbleed 1	Postbleed 2	Prebleed	Postbleed 1	Postbleed 2
1:200	0.055	1.13	1.195	0.053	1.164	1.183
1:1000	0.043	1.082	1.161	0.044	1.152	1.15
1:5000	0.053	0.982	1.024	0.041	1.021	1.126
1:25000	0.041	0.614	0.646	0.041	0.574	0.902
1:125000	0.04	0.195	0.827	0.041	0.186	0.389
1:625000	0.04	0.074	0.074	0.041	0.071	0.124
1:3125000	0.041	0.045	0.049	0.039	0.051	0.055
Background	0.042	0.04	0.041	0.041	0.04	0.043

Coated protein	XXX-XXx					
Animal	XXX			XXX		
Bleed	Prebleed	Postbleed 1	Postbleed 2	Prebleed	Postbleed 1	Postbleed 2
1:200	0.053	0.99	0.962	0.05	0.99	0.965
1:1000	0.041	0.913	0.922	0.041	0.924	0.933
1:5000	0.041	0.847	0.933	0.043	0.803	0.917
1:25000	0.04	0.58	0.846	0.035	0.455	0.736
1:125000	0.037	0.182	0.342	0.039	0.137	0.298
1:625000	0.04	0.071	0.104	0.04	0.065	0.106
1:3125000	0.043	0.044	0.053	0.04	0.045	0.053
Background	0.04	0.041	0.043	0.039	0.045	0.04

**Approved by Applied StemCell, Inc. 521 Cottonwood Dr, Suite #111
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Appendix:

Pictures of the ELISA plates