

Appendix A: NCI and Abcam - Project Detail and Timeline

Average Delivery Timeline	Abcam Responsibilities	NCI Responsibilities
	1. Peptide preparation and conjugation	OR 1. Provide immunogen and screening materials.
3 months	2. Immunize two (2) rabbits*. Evaluate the antisera titer by ELISA. Send pre and test bleeds to NCI for additional assays. *(or 3 rabbits for PTM projects)	
2 weeks		3. Conduct additional assays including all “end application assays”. Discuss results with Abcam and select one (1) rabbit for splenectomy and fusion.
1 week	4. Arrange the final IV boost of the selected rabbit 5. Perform splenectomy and isolate lymphocytes from the spleen	
5-6 weeks	6. Conduct fusion of 40 x 96-well plates. Store all remaining lymphocytes in liquid nitrogen for up to 3 months after the termination of the project.	
	7. Conduct ELISA screen on all 96-well plates against the screening antigen. Expand all positive clones into 24-well plates and conduct confirming and differential ELISA screen.	
	8. Identify and send one (1) ml of all confirmed, ELISA positive hybridoma supernatants (typically 10-100 depending on the antigen and anti-sera titer) to NCI with ELISA datasheet.	
Up to 2 weeks		9. Conduct additional assays including all “end application assays” and rank up to ten (10) hybridomas
Up to 1 week	10. Re-confirming screening with ELISA. Freeze away two (2) sets per positive clone: for revival and for recombinant IgG cloning.	
4-6 weeks	11. Subclone the hybridomas by cloning the corresponding IgG heavy chain and light chain cDNAs from the top three (3) multiclones into a mammalian expression vector. Perform pairwise transient expression of the cloned heavy and light chains from each multiclone in a mammalian 293 derived cell line. Test the expressed recombinant antibodies by standard ELISA to determine the heavy and light chain pair with the best activity.	
	12. Send one (1) ml of culture supernatant to NCI for additional assays.	
Up to 2 weeks		13. Conduct additional assays including all “end application assays” and select final antibody for delivery.
4~6 weeks	14. Bulk produce the recombinant antibody through transient expression in a mammalian 293 derived cell line. Deliver 2 mg of Protein A purified recombinant antibody.	