



Polyclonal antibody technical services

Project Report

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Project number: WG-00044P**1. Project information**

Customer unit: Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences	Customer name: Hu Hongli
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Project start time: 2014/6/19	Project end time:
Species name: Homo sapiens (human)	Gene name: p-RNF168 (p-S60)
Protein size:	NCBI accession number: 165918
Preparation route: phosphorylation	Purification method: antigen affinity purification
Antibody concentration of rabbit number: E1772P 0.52mg/ml E1772C 2.48mg/ml E1774P 0.64mg/ml E1774C 1.63mg/ml	Rabbit number and antibody concentration: E1773P 0.77mg/ml E1773C 3.57mg/ml
Project Overview: Synthetic modified peptides and control peptides were conjugated to Keyhole Limpet Hemocyanin (KLH) via sulfhydryl groups. Four rabbits were immunized with the modified peptide conjugate to generate polyclonal antibodies. Electronic Report: 1. ELISA data and antibody concentration analysis. 2. Peptide synthesis characterization data. 3. Dot blot results confirming immunogen detection. Physical Deliverables: 1. 200 µL pre-immune negative serum. 2. 2 mL rabbit polyclonal antiserum (ELISA titer $\geq 1:20,000$) 3. ≥ 1 mg unconjugated peptide. 4. ≥ 2 mg antigen-affinity purified polyclonal antibody: Concentration: ≥ 1 mg/mL., dot-blot analysis confirmed antibody specificity with a detection limit of 100 ng for the target hapten peptide.	
Advised Option: Modified peptide 56-69aa (p-S60):RRRSS (p) WTRYHTRRN-C The corresponding peptide is 56-69aa: RRVSSWTRYHTRRN-C	

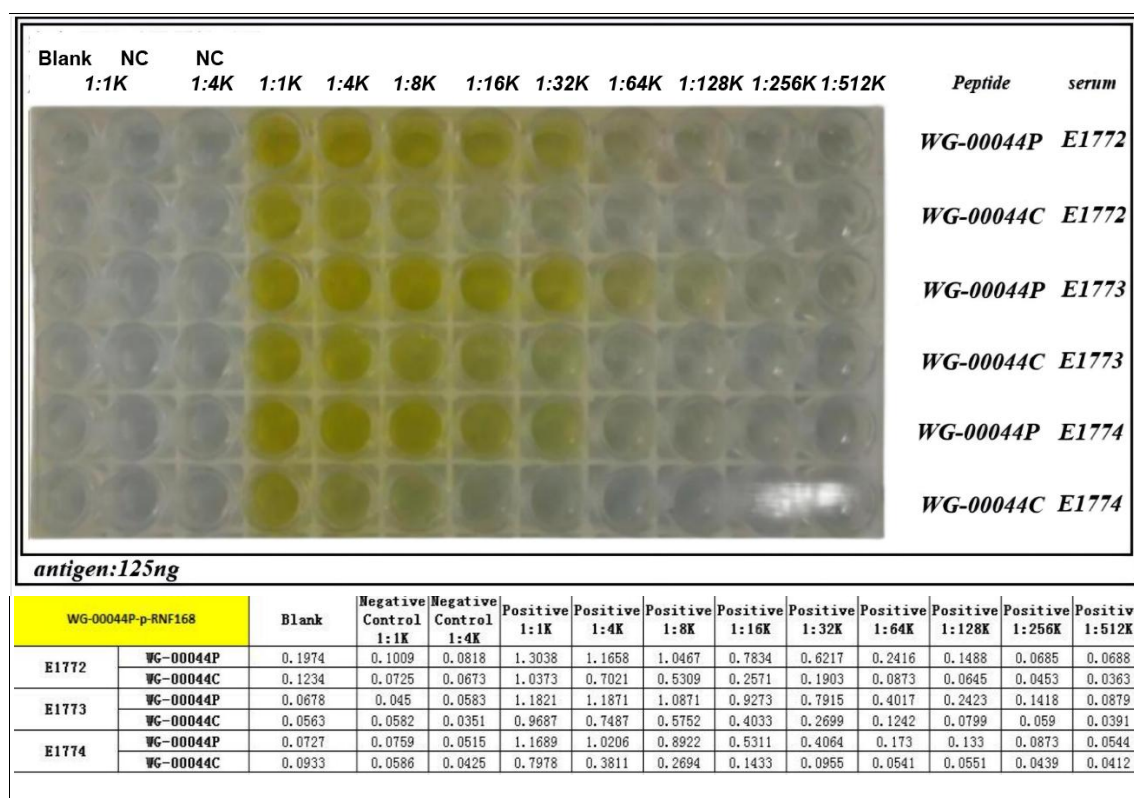
2. Project report content**2.1 Antigen preparation****Immunogenic proteins**

The modified peptide RRVSS (p) WTRYHTRRN-C was used for modification coupling KLH, and 4 rabbits were immunized with the modified peptide

2.2 Immune process

Number of immunizations	The immune cycle	Immunity duration	Immunizing dose	Immunologic adjuvant	Immunocompromised animals
First immunization	Zero days	2014/7/11	1.4 mg	complete Freund's adjuvant	Healthy
Second immunity	12 days	2014/7/23	0.7 mg	Incomplete Freund's adjuvant	Healthy
Third immunity	33 days	2014/8/13	0.7 mg	Incomplete Freund's adjuvant	Healthy(E1775 deaths, Received booster immunization.)
Fourth immunization	54 days	2014/9/3	0.7 mg	Incomplete Freund's adjuvant	Healthy
Blood was collected from immunized animals	66 days	2014/9/15			Blood collection was normal

2.3 anti-serum ELISA detection data



2.4 Antibody purification

1) Antiserum purification

Antibodies were concentrated following affinity purification of serum using modified peptides and control peptides as ligands:

E1772p: Concentration 0.52 mg/ml

E1772c: Concentration 0.48 mg/mL

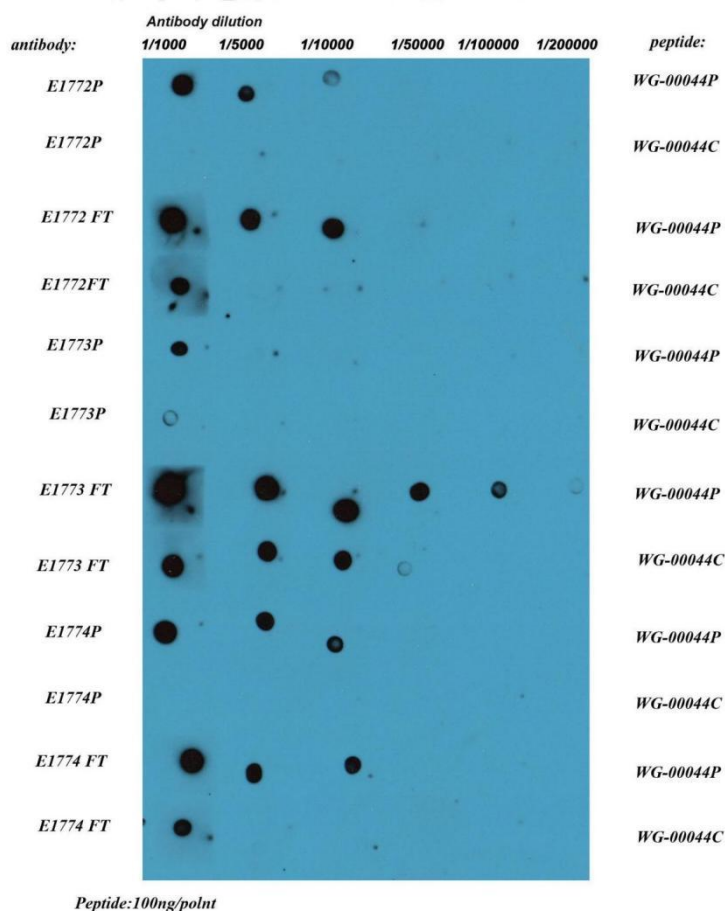
E1773p: Concentration 0.77 mg/mL

E1773c: Concentration 3.57 mg/mL

E1774p: Concentration 0.64 mg/mL

E1774c: Concentration 1.63 mg/mL

2.5 Western blot detection results for the recombinant antigen and endogenous protein



Note: The amount of polypeptide coating was 100ng, and the antibody dilution ratio was 1:1000, 1:5000, 1:10000, 1:50000, 1:100000, 1:200000.

Analysis of results: Antibody concentrations for E1770P, E1773P, and E1774P were suboptimal. Dot blot analysis revealed moderate titers with good specificity for E1772P and E1774P antibodies, while E1773P exhibited suboptimal titer. Elevated antibody titer was observed in the flow-through fraction, though with moderate specificity.

3. Precautions for antibody use and storage

WB dilution: 1/500-1/1000

Storage conditions: -20°C storage, avoid repeated freezing and thawing

Buffer system: PBS, 50% glycerol, pH7.3