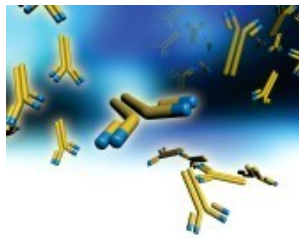


Custom Antibodies Services GeneCust Europe



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Polyclonal Antibody Service

Catalogue #	Description	Timeline	Price
Polyclonal package 1 (From protein to antisera)	<p>Customer provides antigen protein Protein will be checked by SDS-PAGE Immunize two New Zealand rabbits Titer the immunization by ELISA Post-bleed collection Delivery : 1-2ml pre-immune sera 40-60ml antisera ELISA result project datasheet (ELISA result)</p>	10-12 weeks	530 €
Polyclonal package 2 (From protein to antigen purified antibodies)	<p>Customer provides antigen protein Protein will be checked by SDS-PAGE Immunize two New Zealand rabbits Titer the immunization by ELISA Post-bleed collection Antigen affinity purification Delivery : 1-2ml pre-bleed 2-5mg affinity purified antibody Project datasheet including ELISA data</p>	10-12 weeks	660 €
Polyclonal package 3 (From peptide design to antisera)	<p>Customer provides antigen peptide information (or we provide candidate sequences for peptide antigen and discuss with customer. Customer will make his final decision) Synthesize one peptide (12-22aa, purity>85%) Conjugate the peptide to carrier protein (KLH, BSA or OVA) Immunize two New Zealand rabbits Titer the immunization by ELISA Post-bleed collection Delivery : 2-3mg antigen peptide 1-2ml pre-bleed 40-60ml post-bleed Project datasheet including ELISA data</p>	12-15 weeks	730 €
Polyclonal package 4 (From peptide design to antigen purified antibodies)	<p>Customer provides antigen peptide information (or we provide candidate sequences for peptide antigen and discuss with customer. Customer will make his final decision) Synthesize one peptide (12-22aa, purity>85%) Conjugate the peptide to carrier protein (KLH, BSA or OVA) Immunize two New Zealand rabbits Titer the immunization by ELISA Post-bleed collection Antigen affinity purification Delivery : 2-3mg antigen peptide 1-2ml pre-bleed 2-5mg affinity purified antibody Project datasheet including ELISA data</p>	12-15 weeks	830 €

Catalogue #	Description	Timeline	Price
Polyclonal package 5 (From plasmid to antisera)	<p>Customer provides expression vector Express and purify the antigen protein Immunize two New Zealand rabbits Titer the immunization by ELISA Post-bleed collection</p> <p>Delivery : 1mg antigen protein 1-2ml pre-bleed 40-60ml post-bleed Project datasheet including ELISA data</p>	12-15 weeks	990 €
Polyclonal package 6 (From plasmid to antigen purified antibodies)	<p>Customer provides expression vector Express and purify the antigen protein Immunize two New Zealand rabbits Titer the immunization by ELISA Post-bleed collection Antigen affinity purification</p> <p>Delivery : 1mg antigen protein 1-2ml pre-bleed 2-5mg affinity purified antibody Project datasheet including ELISA data</p>	12-15 weeks	1120 €
Polyclonal package 7 (From cDNA to antiserum)	<p>Customer provides protein information We clone the gene and construct the expression vector for fusion protein Express and purify the antigen protein Immunize two New Zealand rabbits Titer the immunization by ELISA Post-bleed collection</p> <p>Delivery : Recombinant vector 1mg protein 1-2ml pre-bleed 40-60ml post-bleed Project datasheet including ELISA data</p>	14-16 weeks	1520 €
Polyclonal package 8 (From cDNA to antigen purified antibody)	<p>Customer provides gene and protein information We clone the gene and construct the expression vector for fusion protein Express and purify the antigen protein Immunize two New Zealand rabbits Titer the immunization by ELISA Post-bleed collection Antigen affinity purification</p> <p>Delivery : Recombinant vector 1mg protein 1-2ml pre-bleed 2-5mg affinity purified antibody Project datasheet including ELISA data</p>	14-16 weeks	1650 €

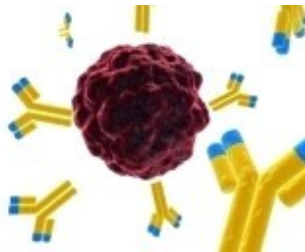
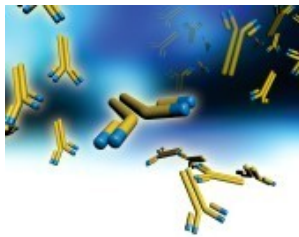
Catalogue #	Description	Timeline	Price
Polyclonal package 9 (Phospho- specific polyclonal antibodies)	<p>Customer provides antigen peptide information (or we provide candidate sequences for peptide antigen and discuss with customer. Customer will make his final decision)</p> <p>Synthesize one peptide (12-22aa, purity>85%) Conjugate the peptide to carrier protein (KLH, BSA or OVA) Immunize two New Zealand rabbits Titer the immunization by ELISA Post-bleed collection Antigen affinity purification</p> <p>Delivery : 2-3mg peptide/EA (non- & phospho- peptide) 1-2ml pre-bleed 2-5mg antibody against unphospho-peptide 2-5mg phospho-specific antibody Project datasheet including ELISA data</p>	13-16 weeks	1650 €
Polyclonal package 10 (Western guaranteed polyclonal antibodies)	<p>Customer provides antigen peptide information (or we provide candidate sequences for peptide antigen and discuss with customer. Customer will make his final decision)</p> <p>Synthesize one peptide (12-22aa, purity>85%) Conjugate the peptide to carrier protein (KLH, BSA or OVA) Immunize two New Zealand rabbits Titer the immunization by ELISA Post-bleed collection Antigen affinity purification Western blot confirmation</p> <p>Delivery : 2-3mg antigen peptide 1-2ml pre-bleed 2-5mg affinity purified antibody Project datasheet including ELISA and Western blot data</p>	15-18 weeks	1820 €
Polyclonal package 11 (Western and IHC guaranteed polyclonal antibodies)	<p>Customer provides antigen peptide information (or we provide candidate sequences for peptide antigen and discuss with customer. Customer will make his final decision)</p> <p>Synthesize one peptide (12-22aa, purity>85%) Conjugate the peptide to carrier protein (KLH, BSA or OVA) Immunize two New Zealand rabbits Titer the immunization by ELISA Post-bleed collection Antigen affinity purification WB and IHC confirmation</p> <p>Delivery : 2-3mg antigen peptide 1-2ml pre-bleed 2-5mg affinity purified antibody Project datasheet including ELISA, Western blot and IHC data</p>	15-18 weeks	2500 €

Monoclonal Antibody Service

Catalogue #	Description	Timeline	Price
Monoclonal package 1 (From protein to ascites purification)	<p>Customer provides antigen protein Protein will be checked by SDS-PAGE Immunize four mice (Balb/C) Titer the immunization by ELISA Hybridoma fusion and cloning Screening: at least one hybridoma clone which is positive to antigen (total five hybridoma clones). IgG isotype determination Ascites are prepared from two hybridoma clones which customer selects. Antigen affinity purification Delivery : 50ul pre-bleed (1:10 dilution) 2-5 hybridoma clones 2-5mg purified antibody from two hybridoma clones Project datasheet including ELISA, and isotype determination data</p>	18-24 weeks	3960 €
Monoclonal package 2 (From peptide design to ascites purification)	<p>Customer provides antigen peptide information (or we provide candidate sequences for peptide antigen and discuss with customer. Customer will make his final decision) Synthesize one peptide (12-22aa, purity>85%) Conjugate the peptide to carrier protein (KLH, BSA or OVA) Immunize four mice (Balb/C) Titer the immunization by ELISA Hybridoma fusion and cloning Screening: at least one hybridoma clone which is positive to antigen (total five hybridoma clones). IgG isotype determination Ascites are prepared from two hybridoma clones which customer selects. Antigen affinity purification Delivery : 2-3mg peptides 50ul pre-bleed (1:10 dilution) 2-5 hybridoma clones 2-5mg purified antibody from two hybridoma clones Project datasheet including ELISA, and isotype determination data</p>	20-26 weeks	4130 €
Monoclonal package 3 (From plasmid to ascites purification)	<p>Customer provides expression vector Express and purify the antigen protein Immunize four mice (Balb/C) Titer the immunization by ELISA Hybridoma fusion and cloning Screening: at least one hybridoma clone which is positive to antigen (total five hybridoma clones). IgG isotype determination Ascites are prepared from two hybridoma clones which customer selects. Antigen affinity purification</p>	20-26 weeks	4450 €

Catalogue #	Description	Timeline	Price
	<p>Delivery : 1mg protein 50ul pre-bleed (1:10 dilution) 2-5 hybridoma clones 2-5mg purified antibody from two hybridoma clones Project datasheet including ELISA, and isotype determination data</p>		
<p>Monoclonal package 4 (From cDNA to ascites purification)</p>	<p>Customer provides gene and protein information We clone the gene and construct the expression vector for fusion protein Express and purify the antigen protein Immunize four mice (Balb/C) Titer the immunization by ELISA Hybridoma fusion and cloning Screening: at least one hybridoma clone which is positive to antigen (total five hybridoma clones). IgG isotype determination Ascites are prepared from two hybridoma clones which customer selects. Antigen affinity purification Delivery : recombinant vector 1mg protein 50ul pre-bleed (1:10 dilution) 2-5 hybridoma clones 2-5mg purified antibody from two hybridoma clones Project datasheet including ELISA, and isotype determination data</p>	20-26 weeks	4620 €
<p>Monoclonal package 5 (Phospho- specific monoclonal antibodies)</p>	<p>Customer provides antigen peptide information (or we provide candidate sequences for peptide antigen and discuss with customer. Customer will make his final decision) Synthesize one peptide (12-22aa, purity>85%) Conjugate the peptide to carrier protein (KLH, BSA or OVA) Immunize six mice (Balb/C) Titer the immunization by ELISA Hybridoma fusion and cloning Screening: at least one hybridoma clone which is positive to antigen (total 2 hybridoma clones). IgG isotype determination Ascites are prepared from two hybridoma clones which customer selects. Antigen affinity purification Delivery : 2-3mg peptide including non- and phosphor-peptide 50ul pre-bleed (1:10 dilution) 2 hybridoma clones specific for phosphor-peptide 2-5mg purified phosphor-specific antibody from two hybridoma clones Project datasheet including ELISA, and isotype determination data</p>	20-26 weeks	5280 €

Catalogue #	Description	Timeline	Price
Monoclonal package 6 (Western guaranteed monoclonal antibodies)	<p>Customer provides antigen peptide information (or we provide candidate sequences for peptide antigen and discuss with customer. Customer will make his final decision)</p> <p>Synthesize one peptide (12-22aa, purity>85%)</p> <p>Conjugate the peptide to carrier protein (KLH, BSA or OVA)</p> <p>Immunize six mice (Balb/C)</p> <p>Titer the immunization by ELISA</p> <p>Hybridoma fusion and cloning</p> <p>Screening: at least one hybridoma clone which is positive to antigen (total five hybridoma clones).</p> <p>IgG isotype determination</p> <p>Ascites are prepared from two hybridoma clones which customer selects.</p> <p>Antigen affinity purification</p> <p>Western blot for QC.</p> <p>Delivery :</p> <p>2-3mg peptides</p> <p>50ul pre-bleed (1:10 dilution)</p> <p>2-5 hybridoma clones</p> <p>2-5mg purified antibody from two hybridoma clones</p> <p>Project datasheet including ELISA, Western blot and isotype determination</p>	20-26 weeks	5780 €



Polyclonal antibody production process

Ready	Animals : two New Zealand rabbits
	Adjuvant : First injection with complete Freund's adjuvant, then incomplete Freund's adjuvant.
	Immunogen : Protein or KLH-coupled peptide; each injection : 100 to 200 µg of immunogen
methods	Immunisations : immunogen diluted with saline buffer, and mixed with the appropriate adjuvant (mass ratio is 1:1). The emulsion is injected to rabbits by subcutaneous injection around the shoulders and intramuscular injection back thighs. Each injection takes about 1/4 immunogen. Then immunogen can be persistent so as to enhance the immune response.
	Blood : rabbit ear artery for blood serum, precipitation at room temperature overnight
Process	Day00 : Serum collected 2ml (0.5-1ml pre-immune serum)
	Day01 : 1 st -immunization, complete Freund's adjuvant 200µg antigen
	Day15 : 2 nd -immunization, complete Freund's adjuvant 200µg antigen
	Day29 : 3 rd -immunization, incomplete Freund's adjuvant 100µg antigen
	Day35 : Serum collected 2-3ml ,ELISA and WB test
	Day36 : 4 th -immunization, incomplete Freund's adjuvant 100µg antigen
	Day43 : 5 th -immunization, incomplete Freund's adjuvant 100µg antigen If Elisa test is negative, 2-3ml bleeding for ELISA and WB test
	Day50 : 6 th -immunization, incomplete Freund's adjuvant 100µg antigen If test is still negative, we need to discuss about project
	Day57 : 7 th -immunization, incomplete Freund's adjuvant 100µg antigen
	Day64 : last bleeding
	Day65 : serum purification, final tests
Day69 : Delivery	

Note:

1. Process is 9-weeks immunization and bleeding protocol. Upon first test results we should be able to determine if project will continue or terminate. Based customer requirements, we can extend protocol with additional fee.
2. Synthesis of peptide will include a Cys for coupling in C or N-end !
3. Peptide synthesis takes about 2 weeks

Monoclonal antibody production process

Step1: antigen preparation

Protein : customer should provide 5mg antigen for immunization and 1mg for ELISA test.

Peptide: design & Synthesis.

Step2: Immunization (8-10 weeks)

Preparation of 4 BALB/C mice, 6-weeks-old

Process :

	Process
Day 0	50ul pre-immune sera and 1 st -immunization with CFA
Day 21	2 nd -immunization with IFA
Day 35	3 rd -immunization with IFA
Day 45	serum collected for ELISA test
We will provide ELISA data by e-mail	

If we get high serum titer (at least 1:10,000 dilution), we will proceed with step 3.

Step3: Hybridoma development and screening (2-3 weeks)

	process
Day 50	Strengthen-immunization for 2 mice with PBS
Day 54	collection of positive serum; then we perform two hybridoma fusions based on highest antigen binding titer
Day 64-68	ELISA test for cell culture supernatant and selection of positive clones; Identification of positive antibodies' subtypes
Day 69-83	Provide ELISA data to customer for control and we can ship 200ul culture supernatants for each positive hybridoma's for testing. We will keep 10-20 ELISA positive clones and keep all the positive clones 2 weeks for message/results/selection.
Day 84	Customer selection/validation

Some positive clones will be lost during subcloning and expanding process. So we have to select 10-15 clones at the beginning to guarantee 5-10 clones at final.

Step4: Subcloning (4-5 weeks) of selected clones (5-10 clones)

Day 61	1 st -subcloning
Day 71	ELISA test of cell supernatants and selection of positive clones
Day 72	2 nd -subcloning
Day 82	ELISA test of cell supernatants and selection of positive clones
Day 83	3 rd -subcloning
Day 83-day 84	ELISA test of cell supernatants and selection of positive clones
Day 84-90	Choose 1 subclone with the highest ELISA titer for each positive hybridoma

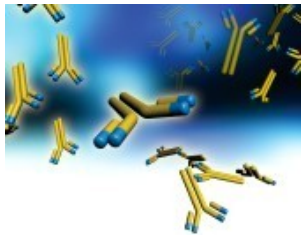
Monoclonal antibody production process

Step5: Expand hybridoma and monoclonal antibody production (5-10 clones) (4-5 weeks)

Day 91	for each clone : injecting 2 mice with liquid paraffin
Day 91-98	culture
Day99	ELISA test for cell supernants; Identification of all positive clones
Day92	Injection of the hybridoma to mice intraperitoneally
Day100-106	Ascites collection
Day107-114	Antibody purification by protein A/G and ELISA test

Step6: Identification

ELISA>1:80000 (Affinity purified antibody)



GeneCust

Custom Services for Research



Thank you for your time.