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Custom Antibody Service FAQs

Version 5.1, Revision 2011-08-18

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Custom Monoclonal Antibody Production FAQ

What services do you provide?
Our services include:
» monoclonal antibody production
» Polyclonal Antibody production
» Large scale monoclonal antibody production from hybridoma
» Monoclonal Hybridoma Library Development
If additional service is required, please send your suggestion to **custom@Biomatik.com**

Do you develop antibodies for customers outside of USA/Canada?
Yes, we do, and Biomatik has been serving worldwide customers.

Do you have any guarantees?
In general, Biomatik guarantees 1:32,000 Elisa titer for purified polyclonal antibody and 1:80,000 for monoclonal antibody. Biomatik provides the best available Elisa guarantee in the industry.

Which antibodies are better: polyclonal or monoclonal?
There are advantages and disadvantages to both. Polyclonal antibody production is less expensive. It is technically easy and fast, but it gives a limited amount of antibodies with multiple specificities. Monoclonal antibody production takes more time and is more complicated, but it gives a limitless amount of antibodies with single epitope specificity. These antibodies provide clear signal and less background in their usage.

Could Biomatik help with an immunization strategy for my project?
Yes, we have extensive experience with various immunization strategies and screening strategies. We can help you determine the best strategy for your project. Since antigen preparations can take some time, talk to us before you start your project. It is never too early to talk to us and develop antibody development strategies. In depth discuss before and during antibody development is a key to our collective success. Please feel free to contact us.

Who owns the rights of the hybridoma clones and antibodies?
The hybridomas and antibodies are exclusively your property, unless other arrangements are made. We welcome collaborative development efforts. Please feel free to contact us regarding these special research arrangements.

How long the titer will remain after last booster? How to reduce the background and to have a better chance to see reaction with native protein? Usually, the titers of the antibody will keep at higher level from day 10 for 6-8 weeks from last booster. The reboot on the animal should increase the titers of the antiserum at this moment. However, it may also increase the backgrounds in some cases. Actually, it's very unpredictable, whether a designed peptide antibody will be against the native protein. The reason is that native protein may be quite different from peptides in many ways like three dimensional folded structure, glycolysation and surface lipids, which all could mask the epitope. Please bear in mind, the immune system only responses to the epitope, but not the sequence molecule. Besides, based on our experiences, some antibodies never works in immunohischemistry, even the antibodies had been confirmed really against the native proteins with other immunodetecting methods. This could be resolved by using the mix antigens immunization of the animals. For example, with the mixture of two peptides, one is come from the N-terminal and another one C-terminal in same protein. The affinity-purification of the crude serum also improves the property of the antibody with relatively increasing the concentration of specific IgGs. Some other clues to consider: We would like to suggest you to change some experimental conditions first, including lower antibody concentration, lower incubating temperature (4ºC or 14ºC), higher detergent concentration, blocking the membrane with higher concentration of BSA or blocking the membrane with donkey and horse serum, and the second antibody from other companies or species. You may try to affinity purify the antiserum by the column coupled with specific peptides. In our experience, most customers are very pleased with their affinity-purified antibodies, because the purified antibody could remove the background effectively. However, some customers are not so lucky, especially for the proteins came from plants or bacterial. If your protein is from plants or bacterial, you may want to try the different eluting fractions from affinity column. This method works well for some our customers.

What happens if there are no antibodies or ELISA is negative?
We recommend ELISA analysis on the first bleed to determine antibody titer. Most animals will respond by this time. A decision can then be made and the protocol modified or terminated. Antibody testing is done by ELISA using the free peptide or protein coated ELISA plates. Antibodies to the carrier proteins are not detected this way. If results are negative, testing is repeated at no additional cost on next bleed. If there are no signs of antibody by ELISA and another technique (Western, IHC), it may be necessary to terminate the project or include changes in immunization protocol. Most custom antibody work is performed on best-effort basis. There are number of reasons when antigens fail to induce antibody response. It may be due the poor antigenicity of an antigen, conservation of peptide sequence in a given species. Some factors are not in our control and we can not alter the nature of antigens or antibodies. In such cases, we offer to use other antigens. In case of peptides, you may elect to have another peptide made and we will make antibodies at no extra cost. In this case, we will only add the cost of making the new peptide and its conjugation. No scientist can predict functionality of antibodies. In many cases, antibodies may not work in all techniques. A given antibody may not work in blotting and be still useful for ELISA or IHC or IP's or vice versa. This is particularly true for anti-peptide antibodies. In some cases high titer antibodies generated against the peptide may not recognize the full-length protein in Western or fail to immunoprecipitate the antigen or may not work in immunohistochemistry.

How clones are identified?
Our initial screening is by ELISA. Additional screenings and assays are available. We have extensive experience in alternative screening assays. Please inquire regarding your specific project needs.

What is the chance that your customer-designed antibody can recognize native protein? How to get a single band in Western Blot test with your antibody?
It is hard to predict if a designed peptide antibody will be against native proteins. Most customers design at least two peptides to generate the antibodies against the same protein.
It's very hard to identify the specific bands with too much background in Western blots. We would like to suggest you to test the antibodies with the immunoprecipitation (IP). Don't worry about the background, as long as the target bands coming out. The most backgrounds can be removed by the affinity-purification of the crude antiserum, and the purified IgG can be used in most immunodetecting experiments. So, higher concentration of the antiserum is recommended in the IP, (1:25, 1:50 or 1:100).
The idea is that the target protein is firstly pulled down by the IgG in the antiserum, and then the target proteins can be probed by

the anti-tag antibody or the antiserum itself. This experiment can remove most backgrounds. If the protein is cloned from the expression of the constructs, we would strongly recommend you to use the monoclonal anti-tag antibody as the primary antibody to do the Western Blot, the results will be much better than the antiserum.

Can you give me "approximate" antibody concentrations for the common production systems?
Most spinners and fermentors are usually in the ug/ml range. With a lot of optimization work some people have gotten to the low mg/ml range (1 to 3 mg/ml). With ascites you should get the mid to high (3-10 mg/ml). There are exceptions to all of these ranges that are higher or lower. Also some sub classes seem to be higher or lower depending on how they are assayed.

Custom Polyclonal Antibody Production FAQ

Let me know about Biomatik's immunization protocol?
Biomatik uses our standard protocol in developing polyclonal antibodies. Our standard protocol contains 8 boosts in approx. 70-day period. Express 35-day immunization protocol is available upon request; we have documented a greater than 95% success rate using our protocol.

Will the titer of antibody increase with additional immunizations?
For rabbit projects, the titers remain relatively stable level after standard protocol.
Additional immunizations are not likely to increase titer. It helps maintain antibody titers rather than to increase them.

How much serum will I receive from polyclonal antibody production?
Approx. 60-80 ml of antiserum from two rabbits. We can produce polyclonal antibodies in a few to several hundred rabbits per run to meet your specific needs.

What concentration of antibody can be expected?
Concentration of purified antibody can vary. Typically 0.5mg/ml to 1.5mg/ml, advise us know if you have specific concentration requirement.

What is sodium azide and should I add it to the antiserum?
Sodium azide is a preservative that prevents bacteria from growing in the serum (bacteria can produce proteases that denature any proteins in the serum, including antibodies). For cell culture usage, sodium azide should not be added to the serum. We typically recommend adding sodium azide unless the serum is going to be used directly in cell culture applications. If you already have sodium azide in the serum, it can be easily removed by using a dialysis membrane with a molecular weight cutoff between 12,000 and 14,000.

How will the serum be shipped?
Serum is typically shipped on ice packs. If sodium azide has been added to the serum, the antibodies can remain at room temperature for at least a week.

How should I store the serum / antisera?
For short-term storage of the working antibody or of serum that has recently arrived, 2-3 weeks at 4°C is recommended. For long-term storage, we recommend storing the serum vials at -20°C, which is sufficient for several years. Storage at -80°C is fine also, but not necessary except for very long periods of time. The purified antibody should be aliquot into working quantities and

stored according to the guidelines above. The biggest threat to antibody stability is repeated freeze/thaw cycles. To avoid this, we recommend storing ready-to-use aliquots of the antibody and thawing aliquots only as they are needed.

Antigens FAQ

What antigens can be used to make antibodies?
We develop antibodies to a wide variety of antigens including, but not limited to proteins, synthetic peptides, cell preparations, DNA, small molecules. Please inquire if you have any questions. However, no biohazardous material can be accepted.

Should I generate antibodies against a full-length protein or a peptide?
Generating antibodies against a full-length protein will provide a pool of antibodies against multiple epitopes from the protein, thereby maximizing the probability of recognizing the endogenous protein in the target assay. However, this larger pool of antibodies does increase the possibility that some of those antibodies will cross-react with other proteins in the assay.
Immunizing with a peptide sequence, by contrast, allows the serum to be affinity purified against the peptide sequence, thereby permitting isolation of antibodies that are highly specific to the target protein. The only potential downside to this approach is that there is a risk that the chosen sequence won't correspond to an exposed region of the native protein.

What options to consider in the development of peptide Immunogens?
The following information is offered by Biomatik to our clients requesting background on the development of effective peptide immunogens. These comments are a brief summary of our collective experience in this area.
The design of an effective peptide immunogen is a complex subject, and the following comments are meant to provide a quick introduction to the key issues most commonly encountered in the design of a peptide immunogen.

General points that should be considered:
Peptide Length: 12aa – 22aa is mostly recommended. Around eight to ten residues seem to be the smallest peptide most people try to use. Peptides of 15 to 20 residues are the most commonly used in developing antisera for western blot, histology and immuno-affinity or precipitation if there are no constraints in selecting such peptides. Longer peptides (>20 aa) can be used but it increases the cost. It is generally not a good idea to chose peptides <8 aa unless there are valid reasons for it such as potential sequence homology with a related family member or other proteins. Shorter peptides (<8 aa) may present limited number of epitopes.
Conjugation to a Carrier Protein: The immune response to a peptide conjugated to a carrier protein is, with few exceptions, going to be stronger than will be seen for the peptide alone.
Carrier Protein: The most commonly used carrier today is KLH (Keyhole Limpet Hemocyanin) but albumins, generally Bovine, IgG's, PSG (Pumpkin Seed Globulin), and a number of other proteins can and have been used.
Conjugation Chemistry: Where possible, particularly when there is no cystine in the peptide sequence, consider the use of a sulfhydryl cross-linker through a cystine residue added at one end of the peptide. The decision about which end to add the cystine should be based on the ease of production. There are two exceptions to this: very short peptides and peptides from the C- or N-terminal end of the protein sequence. For short peptides, the use of a mixture of both C- and N-terminal linked peptides, or better, two separate programs on using an N-terminal coupling and the other a C-terminal coupling. Conjugations can also be performed using general reagents such as glutaraldehyde, or more specifically, using N-or C-terminal specific cross-linkers. Any of these may also cross-link through any side chains with the appropriate reactivity. For example, N-terminal reagents will react with the primary amine on the lysine side chain. Peptides linked to multiple copies of the carrier through their side chains are generally thought to be less effective as immunogens. Finally, choosing a peptide sequence to improve the likelihood of a clean conjugation to a carrier is usually counter productive.

Should I consider adding a Cysteine in peptides for making antibodies?
All small peptides must be coupled to a carrier protein (KLH, BSA, Ovalbumin, etc) in order to elicit high titer antibodies. Generally, peptides can be coupled to other proteins by utilizing a free NH2 or COOH, or a Cysteine group. Chemical conjugation using Cysteine offers a single point attachment provided there is just one Cys in the sequence (added or part of the native sequence). It is preferable to add Cys at the NH2 terminus if the peptide is internal or it represents the very C-terminus.

This will keep the COOH free (non-conjugated) as it exists in native protein. For peptides representing the very NH2-terminal sequences, Cys should be added at the C-terminus of the peptide. For internal peptides, Cys can be added at either end but it is easier to synthesize peptides containing a NH2-terminal Cysteine. Cysteine can also be used to couple peptides to Sepharose for affinity purification of antibodies. Amino or COOH-conjugation chemistries should be avoided as most peptides contain several NH2 and COOH groups available in a given peptide sequence resulting into multi-point attachment and peptide distortion.

Can I order peptide synthesis at Biomatik?
Yes, Biomatik Custom Services provide all the services required for antibody development including peptide design, synthesis and peptide conjugation to carrier proteins.

What quality control (QC) information do you provide?
All peptides are analysed by MS to confirm the molecular weight. Purity shall be greater than 85% by HPLC.

Can you explain the M+Na and M+K mass peaks in MALDI spectra?
It is very common to see Na (sodium) and K (potassium) adducts in the MALDI spectrum. The sodium and potassium comes from the water used in the peptide solvents. Even distilled and deionized water has trace amounts of sodium and potassium ions, which can never be entirely removed. These become ionized during the MALDI mass spec process and bind to the free carboxyl groups of the peptide. Because there is no water purification system that will remove every single sodium or potassium ion from water, seeing the sodium and potassium adducts at times is very common and unavoidable in MALDI mass spec. This is not an indication that the peptide is not pure, nor should it be confused with an incorrect molecular weight.

How should my peptide be stored?
Lyophilized peptides should be stored away from heat, light and moisture. Under these conditions lyophilised peptides are stable at room temperature for days to weeks, for long term storage, peptides should be stored under the same conditions but at -20 °C.

How about using multiple peptides as antigens?
To make a good antibody, some researchers try to generate the antibodies by different sites on the protein sequence, and ideally select 3 sites, N-terminal, C-terminal and the middle, to do the immunization. Believe or not, the different site peptide will give you different results. Another option is that you may make two peptides and mix them to immunize the same animal.
Actually, both human and rat sequences are good for the antibody production. The antibody raised by one of both sequences will recognize both peptides. Please bear mind, the immune system is only response to the epitope, but not the sequence molecule. Sometimes, the antibody may be only against the peptide but not the native protein, that's because the peptide forms a different epitope after the folding.
The human sequence antibody works in human tissue, so it's for sure the antibody against native protein. In most case, if the antibody is against human protein, it will be also against same protein in other species. This is an advantage of the polyclonal antibody. We had been used a polyclonal antibody from rat sequence to do lots of experiments with human and mouse tissues.

Will I receive peptide at the end of the project?
Yes, you will receive up to 5mg peptide with antibody.

Do you know or suspect the sequence to be highly conserved?
Where data is available, use peptides whose sequences show the largest number of changes between the antigen source species and the planned antibody source species.

Is it necessary to use 95-98% pure peptides for antibody production?

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Although, pure peptides are always better but it is not a requirement to have 90-95% pure peptides for antibody purposes. It is generally more economical to synthesize about 85% pure peptides than to spend lot more to purify peptides. The antibody project is not likely to fail because of the use of 85% pure peptide as opposed to >90% or better purity.

How about coupling peptides to 2 different carriers (KLH and BSA)?
For most anti-peptide antibody purpose, coupling of peptides to just one carrier protein (KLH or BSA) should be sufficient for immunization. Many small peptides will not coat well on ELISA plates. Coupling of peptides to another protein is done to facilitate coating of peptide on ELISA plates. Biomatik uses free peptide for coating and screening antibodies by ELISA. We have developed special technique to coat most peptides to ELISA plates. Therefore, we do not recommend coupling peptides to more than one protein and hence save these expenses.

Is help available from Biomatik for peptide/antigen design?
Yes, we provide free peptide design when you do an antibody development project with us. If you provide us with the amino acid sequence of the target protein, our peptide chemists will analyze your protein sequence and recommend the most immunogenic sequences based on such factors as the hydrophobicity / hydrophilicity and folding characteristics of the protein. In depth discussion in antigen design and screening strategy is the key to success. This service may also be used to pick from a number of peptides, the one or two most likely to illicit an immune response. It will always remain the investigator's responsibility to select the peptide to be used. We have consistently obtained very good results with these recommendations.
Biomatik has analyzed thousands of sequences and assisted researchers in selecting immunogenic peptides. It is very helpful to have additional information as to what regions of the protein (N or C-terminus, or a given domain) to specifically target or avoid. Any potential for cross reactivity with other closely related members of the same family should also be mentioned. A sequence alignment of closely related members is of tremendous help to select specific peptides. All recommended peptides are compared for sequence homology with other proteins by BLAST searches. A final selection is then made with the user input. It is a good idea to provide us with the gene accession number of published sequences or send us the sequence by email. All info shared with us always remains confidential.

Why is conjugation of the peptide to a carrier protein necessary?
Yes, the molecular weight of most peptides is too small to generate an immune response in the animal. Conjugation to a carrier protein such as KLH will not only increase the size of the antigen, but increase its immunogenicity to the peptide epitope as well. KLH, Keyhole Limpet Hemacyanin, is the most commonly used carrier protein because species cross-reactivity is very minimal. It is conjugated to small molecules of haptens or short peptides for antibody production.

Please clarify the KLH-conjugation procedures
Here is the protocol of our KLH-peptide conjugation. Briefly, we weight 15mg KLH reacting with SMCC linker in PBS. After PD-10 column removed unbound SMCC, 15mg of free peptide were added to the SMCC linked KLH for coupling overnight. Finally, the free peptides were removed by exhaustedly dialysis.

Can you conjugate my peptide to a carrier?
Yes. Please consult with our technical staff to recommend peptide synthesis strategies to facilitate carrier coupling and enhance peptide immunogenecity.

What level of antigen purity is required?
Please provide the highest-purity antigen you can. For screening purposes, we will need at least 90% pure antigen (by HPLC, commassie blue staining, silver staining, etc). For immunization, the immunizing antigen may be less than 90% pure. Please inquire if you have purity concerns, we will be able to help.

What buffer should my antigen be in?
We prefer antigens to be in PBS or any non-toxic biological buffer (Tris, Borate, Phosphate, etc) without detergent or Urea. If

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your antigen is in a different buffer, please let us know and we can determine if a buffer change is necessary. It can be sent freezing or at 4°C by customer's discretion.

What concentration should my antigen have?
1mg/ml or higher is preferred. More concentrated antigen is fine. If your antigen has a concentration below 0.2mg/ml, please talk with us.

Can insoluble proteins be used for antibody development?
We have been able to generate antibodies against insoluble proteins using inclusion bodies. In many cases we have observed that a precipitated protein yields better antibody than soluble antigen. The speculation is that the precipitate antigens provide long term antigen depot for continuing stimulation of the immune system and it may also be easier for an antigen presenting cells to engulf. However, it is important that we have a pure, soluble protein for screening purposes. Please inquire with us if you have solubility concerns, we will be more than happy to discuss and provide help.

What if the protein is NOT soluble in regular biological buffers and if it precipitates?
High concentrations of chaotropic agents (8M Urea, GdnHCl, SDS) should be avoided. Often some recombinant antigens will not dissolve in regular buffers and require 4-8M urea etc for keeping these proteins in solution for some client's applications. Try to minimize the concentrations of strong denaturants and detergents as much as possible by dialyzing in buffers containing lower concentration of these components. If all fails, then keep the concentration of protein as high as possible (5-10 mg/ml). It is also possible to inject proteins as precipitates. Just send the protein in buffer containing suspension of proteins. However, these extreme measures (SDS-PAGE, high concentration of urea etc, and precipitates) must be avoided and used only as the last option. Antibody titer may also be compromised.

Can I generate antisera against gel strips containing my protein?
Yes, this method has been used successfully if amount of gel is kept to a minimum. Immunizing with gel bands works well for generating polyclonal antisera against a purified protein. Acrylamide gel, if injected in large amount, is toxic to animals. It is very important to load as much protein as possible on a PREP GEL (no lanes or wells), coomassie blue dye staining, destaining, rinse and then cutting out the most dense portions of the protein band (One gel strip or its equivalent should be enough for the entire protocol). Please avoid freezing or homogenizing the gel strip so that we can aliquot appropriate quantities of the protein for each immunization. With gel bands in particular, it is important to maintain a higher concentration of the protein as outlined above. Biomatik will process your gel slices by mincing and homogenization before injections. Please contact us prior to shipping so that we can prepare to start your project.

Can I supply a fusion protein for immunization?
Yes, we can use the protein prepared by the customer (90% pure, 5 mg/project) for immunizations.

How much antigen do you need?
Amounts of antigen needed to produce good titer antibodies obviously depend upon the antigenicity of a given "antigen" and host species. Bacterial/viral antigens are very often highly immunogenic than mammalian proteins. In our experience, the antigen amounts given below will normally suffice to make good titer antibodies. These estimates are for using up to 2 animals using our standard protocols.
- Purified and recombinant proteins: 500 ug-2 mg.
- Peptide-Protein Conjugates: 2-5 mg.
- Proteins in SDS-PAGE gels: A band that is clearly visible upon staining (500 ug-2 mg).
- Proteins as precipitates: 500 ug-2 mg.

To assure good antibody production, we recommend sending at least 3mg or more of the antigen for a rabbit or chicken project and 5-7 mg for a goat project. It doesn't cost more to inject more. The minimum amount will be 300 ug/per rabbit. We recommend 5mg/ml as a minimum protein concentration and welcome higher concentrations. For peptide antigen, minimum

5mg of peptide conjugated to 2.5 mg of KLH/per rabbit will be needed for immunization.

What role does the adjuvant play and what is the difference between CFA and IFA?
An adjuvant is a substance that, when combined with the antigen, serves to enhance the immune response against the antigen. Freund's adjuvants are the preferred adjuvant of choice for use in antibody production. Complete Freund's Adjuvant is used only for the first immunization and contains mycobacteria, which stimulates the host's immune system. Incomplete Freund's Adjuvant is used for all subsequent immunizations. We recommend the use of Freund's Adjuvant, however, we can accommodate any requirements that you may have for specialized adjuvants.

What is the best method of shipping antigens to Biomatik?
All antigens can be safely shipped depending on the antigen types. Some antigens such as peptide in powder forms or SDS-PAGE gels can be shipped at room temperature. Other antigen (proteins bound to Sepharose gels, precipitates, peptide/protein-conjugate solutions) can be shipped on ice packs. Protein in buffer shall be shipped in dry ice.

Animals FAQ

What Species and animal number are appropriate for antibody production?
Antigen source, its sequence conservation, and antisera volume are the primary factors in choosing a host species and their numbers. For example, antigens purified from rabbits should be injected into goat, g. pig or chicken to produce high titer antibodies. In general, peptide-protein conjugate can be reliably injected into rabbits (even if sequence of the peptide is the same in rabbit). Mammalian protein antigens, that are known to be poor immunogens, could produce high titered antibodies in non-mammalian hosts such as chicken or fish.
Polyclonal antibodies generated in common animals will generally show a variation not only in their titer but also in their quality even if all animals are injected with the same antigen and bled at the same time. Therefore, it is best to include at least 2 or more animals per antibody protocol. It is often recommended to include large number of animals if the desired antibody is expected to distinguish closely related isoforms within or across the species or has other subtle changes (phosphorylated Vs non-phosphorylated). We generally recommend the use of at least 2 animals per protocol in Rabbit, G. Pig. Up to 5-10 animals are usually needed when antibodies are made in mouse/rat to collect sufficient amount of serum.

Antibody Purification FAQ

Can I have my antibody mass produced and purified?
Yes, Biomatik Development Service is a one-stop service offering all the services including mass production (mg to g) & purification of antibodies. We purify antibodies using Protein G or Protein A affinity columns. Antibody concentration is also determined. Give us any possible background with the cell lines, so that we can provide a good estimate of the amount of material to be produced in what time frame.

What is the procedure for phospho-peptide purification? How it works?
We applied the antiserum from rabbit to phosphopeptide column first, then eluted the column and took the eluted fractions to the column of non-phosphopeptide (the flow-through was the used antiserum which was sent to client). The eluted fraction of the non-phosphopeptide column which will mark with anti-non-phosphopeptide, but please be aware, those fractions do not only bind to non-phosphopeptide, but also bind to phosphopeptide. That's why it reacted preferentially with the phospho-form because it's came from the phosphopeptide first. Actually, there should be no real anti-non-phosphopeptide antibody in both

flow-through and eluted fractions.
The flow-through of non-phosphopeptide column did only bind to the phosphopeptide, so it's real anti-phosphopeptide antibody. In our experience, it's better to re-purify this fraction by the phosphopeptide column to get real specific anti-phosphopeptide antibody. The eluted fractions of the re-purification with phosphopeptide column are the final purified antibody. These two should be exactly same.
To get the real anti-non-phosphopeptide antibody, the antiserum must be applied to the non-phosphopeptide column first, and then remove the non-specific IgG by phosphopeptide column.

Does the phospho-antibody work with phosphorylated protein?
We did provide you with antibody ELISA test against phospho-peptide and non-phospho peptide, it will be upon to you to test with corresponding phospho- proteins and non-phospho proteins by your application for example by Western blot. But bear in mind, it's very unpredictable if a designed peptide antibody is against the native protein. There is big gap between peptide and whole protein due to conformation and other structure difference, for example glycolysation. But we have had experience that some of our client's phospho-antibodies did work nicely in immunohischemistry, the antibodies had been confirmed luckily against the native proteins with other immunodetecting methods. So working on conditions with Western blot is the first thing to try.

We would like to suggest you to try some experimental conditions, including enriching the proteins loaded on the gel, purifying the native protein from expressed cells, trying reducing conditions, lower antibody concentration, lower incubating temperature (4ºC or 14ºC), higher detergent concentration, blocking the membrane with higher concentration of BSA or blocking the membrane with donkey and horse serum, and the second antibody from other companies or species.

Why am I seeing multiple or unexpected bands on a Western with my affinity purified antibody?
It isn't uncommon to see multiple bands even when using affinity-purified antibody. This isn't indicative of a problem with the antibody's specificity. Rather, this typically occurs for one of the following reasons:
1. The anti-peptide antibody recognizes a homologous protein in the sample that shares one or more epitopes with the peptide sequence.
2. The native protein is a different molecular weight than previously predicted.
3. The antibody is recognizing either cleaved fragments of the native protein at lower molecular weights or aggregated dimers/trimers of the native protein at higher molecular weights.

Why am I not seeing any bands on my western when assaying with the purified antibody?
In cases where no bands are seen (or an antibody doesn't work in a particular assay), these are the three most common explanations:
1. The peptide sequence corresponds to a non-exposed region of the native protein
2. The protein's conformation in the peptide region differs enough that the antibody has trouble recognizing the native protein.
3. The target protein isn't present in the sample.

How much antibody we will expect to get after affinity purification?
The yield of affinity-purification is very diversity in different rabbit and is depending on the immune response of the individual animal to the antigen. We got 5 to 10 mg of 96-99% purified IgG from 50ml antiserum in most case.

Antibody Products FAQ

How should I store my SDS samples of phosphorylated proteins?
SDS sample cell lysates can be stored at -20°C for short-term use (less than 3 months), but should be kept at -80°C for long-

term storage. The degradation of activated protein samples varies greatly. It depends on the nature of each protein and how much activated protein is within the samples.

Will Biomatik antibodies work on my whole tissue sample lysates?
Biomatik does not test all their antibodies on whole tissue lysates in-house, but many of our antibodies have been used by other customers on whole tissues with great success.

How do I prepare good control cell lysates for this phosphorylated target protein?
Please refer to the Western blot figure on the data sheet for the product that will demonstrate a suitable control cell line and drug treatment. Cell lysate sample preps for activated proteins can vary due to cell number, drug induction timing, cell passage number and storage conditions.
Biomatik products can be expected to perform at optimal levels during their shelf-life period.

If a species is not listed as cross-reactive with a Biomatik catalog antibody on the data sheet, does this mean that the species has not been tested?
We test antibodies on human, mouse and rat species if possible. If we do not obtain positive results in one species, we do not list that species. However, it is possible that the cell line we test may contain a low level of particular protein or the inductions we used do not work in that particular cell line. Therefore, we suggest you compare the peptide sequence around the modification site (5 amino acids on each side) between the species you are studying and the species that the Biomatik antibody was raised against. In most cases, Biomatik antibodies are raised against the human protein sequence and are highly purified using affinity chromatography. Many times if the sequences are identical or have only 1 or 2 amino acid differences in residues other than the phosphorylation site, there is a good possibility the Biomatik antibody will recognize your protein.

How can I ensure reproducible Western results?
There are several possibilities as to why Western-blotting results may seem difficult to repeat. To discover the cause of this situation, consider the following:
The antibodies cannot be re-used after dilution in the primary dilution buffer. Many antibodies have a very low working concentration and it is possible that the concentration will decrease with each successive blot.

Does phospho-specific antibodies react with the non-phospho-specific antigens?
No, there is no cross-reaction with the non-phospho-specific antigens.

In general, will anti-human antibodies react with other species tissues?
If the protein is evolutionally conserved, anti-human antibodies will react with other species tissues. BLAST search gives the data indicating the species in which the protein is expressed.

Need further help? Feel free to contact custom@biomatik.com