



## Antigen Submission Guidelines

Pacific Immunology provides antibody production services using customer-supplied antigens. To maximize the success of your project, please review the following guidelines when preparing your antigen.

### Types of antigens accepted:

We accept soluble proteins, insoluble proteins, fusion tag proteins, gel bands and conjugated small molecules. We can only immunize with viral or bacterial samples that have been killed and are confirmed to no longer be active.

### Quantity of Antigen:

2-3 mg of the antigen is recommended. However, 1-1.5 mg of the antigen will allow for full immunization quantities if no extensions or immunochemistry services are ordered.

Also, the immunogenicity of the antigen is more important than the quantity being immunized. For example, immunizations of 5-10 µg of a very immunogenic antigen can elicit high antibody titers, while immunizations with maximum quantities of a non-immunogenic antigen can elicit low antibody titers.

### Buffer conditions:

Antigens provided in most biological buffers in the range of pH 6-8 are acceptable. Antigens in protein solubilization reagents such as 4-8 M urea can be used, but detergents should be avoided due to interference in the production of a stable adjuvant emulsion and subsequent presentation of the antigen to the immune system.

### Antigen Concentration:

1 mg/ml is an ideal target, but higher concentrations are welcome and most concentrations are okay if we know the approximate measurement. .25 mg/ml is the recommended lower limit.

### General Ordering Information:

Please ship the antigen via FedEx or UPS overnight to:

Pacific Immunology  
1672 Main St. Ste. E #171  
Ramona, CA 92065

- Please send the antigen in a Styrofoam container (no shipping envelopes) with ice packs (dry ice isn't necessary).
- It isn't necessary to aliquot the antigen – this will be done when preparing the first immunization.
- Please enclose a copy of the order form in the box and list the antigen identification, quantity and concentration where indicated.

### Molecular Weight of Antigen:

Antigens weighing less than 6 kD are too small to elicit an immune response by themselves and need to be conjugated to a carrier protein such as KLH. Antigens weighing more than 6 kD can be immunized directly.

### Fusion Tag Protein Considerations:

A protein / fusion tag complex can be used directly for immunizations. However, antibodies will be generated against the fusion tag in addition to the target protein and so may contribute to cross-reactivity in assays.

### Immunochemistry Considerations:

Only soluble proteins can be used to coat microwells for running ELISAs and only proteins that are soluble in PBS can be used to prepare the affinity column for affinity purifications.

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**Gel Band Considerations:**

- Gel bands require that protein concentrations be as high as possible to ensure that maximum quantities of protein are being immunized. .25 mg/ml is the lower limit, with .5 mg/ml or higher being ideal.
- Gel bands should be stained, de-stained, rinsed well and trimmed to avoid as much extraneous material as possible.
- Please do not chop, mash or homogenize the gel bands.
- Please do not elute the protein from the gel bands since this will introduce SDS into the mixture and prevent the formation of an emulsion with the adjuvant. The SDS present in the intact gel bands does not present this problem.
- The protein quantity / concentration can typically be determined by measuring the total protein quantity prior to running the gel and then estimating the concentration of protein in the target band based on its relative size compared to any other bands. For example if 5 mg of total protein is loaded on a preparative gel and it's estimated that the target band accounts for approximately 60% of the total protein bands, then 3 mg of the target protein should be present in the cut-out bands. An exact concentration isn't necessary, only a good estimate.