Invitrogen™
life technologies

Instruction Manual

Custom Antibody

Catalog nos. **M0300, M0353, M0354, M0355, M0356, M0357, M0358, M0342**

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Contents and Storage

Shipping and Storage

The custom antibodies are shipped on dry ice.

Store at 4°C or -20°C.

For long-term storage, aliquot the antibody and store at -20°C or -80°C. Repeated freezing and thawing is not recommended as it may result in loss of antibody activity.

Note: Any remaining peptide after the immunization protocol is supplied as lyophilized material and shipped in glass vials at room temperature. Upon receipt, store peptides in lyophilized form at -20 or -80 ºC for up to a year. For instructions on dissolving the peptide in an appropriate buffer, see page 18.

Contents

At the end of the standard antibody protocol, pre-immune serum, antiserum, test bleed aliquots, and ELISA results of all serum collections are shipped. If antibodies are generated against peptides, any excess peptide is also shipped. The amount of serum shipped with different species is listed in the table below. For interpretation of ELISA results, see next page.

For detailed standard antibody protocols for all species, refer to our web site at www.invitrogen.com/evoquest.

Animal	Antisera*		
	Pre-immune serum	**Antiserum/yolks**	**Test Bleed Aliquots+**
Rabbit	3-5 ml	20 ml (4 week) 20 ml (8 week) 40 ml (10 week)	1 ml (4, 8, 10 week)
Chicken	1 ml	2-10 yolks (3 week) 2-10 yolks (7 week)	5 ml (8 week)
Goat/sheep	2-5 ml	1000 ml plasmapheresis collection	2-5 ml (6, 8, 9, 16 week)
Mouse	0.1-0.25 ml	0.1 ml**	0.1-0.25 ml (7 week)
Rat	~0.5 ml	2 ml**	0.5 ml (7 week)
Hamster	0.1-0.25 ml	2 ml**	0.1-0.25 ml (7 week)
Guinea Pig	1 ml	15 ml**	1 ml (7 week)

*Amount of pre-immune serum, antiserum, and test bleed aliquots are for 1 animal.
**Indicates exsanguination bleed.
+Small aliquots of antiserum supplied for a specific time point.

Introduction

Overview

Introduction

Custom antibodies from Invitrogen are polyclonal antibodies raised in the animal of choice. The antibodies are produced using proven standard protocols.

Applications

The custom polyclonal antibodies are suitable for a wide variety of applications such as:

- Western and dot blotting
- ELISA (Enzyme Linked Immunosorbent Assay)
- Immunoprecipitation
- Immunohistochemistry

Interpreting ELISA Results

ELISA results are shipped with the antibodies and are also available on line at EvoTrack™ (see next page).

The ELISA is performed as described on page 9. The ELISA results are listed as Dilution Titer and ELISA Titer. The options at the end of the standard protocol are listed on the next page.

Dilution Titer

This is the last serial dilution that generates an $OD_{405} \geq 0.2$.

ELISA Titer

The ELISA Titer is the estimated dilution factor derived from non-linear regression analysis of the sigmoidal dilution curve. The interpolated value from the curve that corresponds to an $OD_{405} = 0.2$ is the ELISA Titer.

Continued on next page

Overview, Continued

Note

Please note that Invitrogen does not offer any guarantees regarding the custom polyclonal antibody production service. Some or all animals may fail to give a response to certain peptides. The antibody service is designed to maximize your chances in producing a functional reagent on all levels.

Note the ELISA results included with the antibody reflect how the antibody performs under our experimental conditions. These results may or may not correlate directly to other assays performed with the antibody such as western blotting, immunoprecipitation, or immunohistochemistry. Based on the application for the antibody, the titers can be different and it is difficult to have a best ELISA titer for each application.

We strongly recommend that you validate your antibody by confirming our results using your own methods.

EvoTrack™ Online

EvoTrack™ Online is the new free online project tracking tool offered to all our custom antibody customers and is available on our Web site at www.invitrogen.com. EvoTrack™ Online helps you keep your custom antibody projects on track and view the ELISA results. Securely log on anytime, and find out the status of your project and the expected delivery date. For added convenience you can easily copy ELISA data directly into a spreadsheet saving you time.

Options after Standard Protocol

After confirming the antibody titer at the end of the standard protocol using your own methods, be sure to contact us with a suitable option (listed below) at end of the standard protocol.

For details on each option, contact EvoQuest™ Services at www.invitrogen.com/evoquest.

- Extension (standard boost and antisera collection)
- Maintenance (no boost or antisera collection)
- Terminal Bleed (antisera collection, no boost)
- Aggressive boost (includes a boost with complete Freund's adjuvant followed by a boost with incomplete Freund's adjuvant)
- Double antigen boost (using twice the standard antigen amount followed by a bleed)

Methods

Western and Dot Blot

Introduction

The table below outlines the basic steps of a western blot. Instructions are provided below to perform a western blot using the WesternBreeze® Immunodetection kits available from Invitrogen (page 20).

For other western protocols, refer to Harlow and Lane, 1988.

Step	Description
1	Run an SDS polyacrylamide gel of the purified or partially purified protein or cell lysate with appropriate controls.
2	Transfer the proteins electrophoretically to a PVDF or nitrocellulose (NC) membrane.
3	Probe blot with an appropriate dilution of the custom antibody.
4	Incubate the blot with anti-species IgG secondary antibody conjugated to an enzyme such as alkaline phosphatase or horseradish peroxidase (HRP).
5	Visualize protein using a colorimetric or chemiluminescent detection method.

Materials Needed

You will need the following items:

- Blotted membranes containing the applied antigen
- Appropriate dilution of the custom antibody to detect the applied antigen
- Purified water autoclaved, sterile, or ultra filtered to remove alkaline phosphatase activity from all solutions used in the procedure
- Clean flasks for preparing solutions
- Forceps for manipulating blotted membranes.
- Orbital shaker platform
- WesternBreeze® Immunodetection Kits (page 20)
- X-ray film (Kodak X-OMAT AR films) and autoradiography cassette (for Chemiluminescent detection)

Continued on next page

Western and Dot Blot, Continued

Preparing Solutions

For western blots from Novex® or other mini gels, prepare solutions for nitrocellulose or PVDF membranes as described below for ~60 cm^2 membrane.

Note: The Blocking Solution is used for blocking and as a Primary Antibody Diluent for NC membranes.

Solution	NC Membrane	PVDF Membrane
Blocking Solution Ultra filtered Water: Blocker/Diluent (Part A): Blocker/Diluent (Part B): Total Volume:	 14 ml 4 ml 2 ml 20 ml	 5 ml 2 ml 3 ml 10 ml
Primary Antibody Diluent Typically, primary antibody preparations are diluted 1:1000 to 1:5000	Dilute your primary antibody into 10 ml of NC Blocking Solution prepared above.	Ultra filtered Water: 7 ml Blocker/Diluent (Part A): 2 ml Blocker/Diluent (Part B): 1 ml Total Volume: 10 ml Dilute your primary antibody into this diluent.
Antibody Wash Ultra filtered Water: Antibody Wash Solution (16X): Total Volume:	 150 ml 10 ml 160 ml	 150 ml 10 ml 160 ml
Chemiluminescent Substrate (for chemiluminescence detection only) Chemiluminescent Substrate: Chemiluminescent Substrate Enhancer: Total Volume:	 2.375 ml 0.125 ml 2.5 ml	Use 2.5 ml Chemiluminescent substrate directly from the bottle. **Do not** add the Chemiluminescent Substrate enhancer.

Continued on next page

Western and Dot Blot, Continued

Guidelines

- Avoid touching the working surface of the membrane, even with gloves.
- Use pure water, free from alkaline phosphatase activity. Stored water should be autoclaved or ultra-filtered to remove alkaline phosphatase activity.
- Avoid cross-contamination of system solutions.
- Perform all washing, blocking, and incubating steps on a rotary shaker platform rotating at 1 revolution/second.
- Work quickly when changing solutions as PVDF membranes dry quickly. If PVDF membrane dries, re-wet the membrane with methanol and rinse with water.

Electrophoresis and Transfer

1. Prepare and load your protein samples for electrophoresis on an appropriate gel. Load at least 5-50 ng of your recombinant protein onto the gel.
2. Transfer proteins to nitrocellulose or PVDF membrane using manufacturer's recommendations.
3. After transfer is complete, remove the membrane and prepare the membrane as described below.

Preparing the Membrane

For blots from SDS-PAGE gels to nitrocellulose or PVDF membranes, wash the membranes twice for 5 minutes with 20 ml of pure water to remove gel and transfer buffer components and some weakly bound proteins. Proceed to immunodetection procedure (next page).

If you are using water-washed and dried NC membranes, proceed to immunodetection procedure (next page). For water-washed and dried PVDF membranes, re-wet the membrane in methanol followed by 2 x 20 ml water washes for 5 minutes. Proceed to immunodetection procedure (next page).

For native-PAGE blots, a drying step is recommended to improve protein binding to the membrane. After drying the membrane, re-wet the membrane as described above.

Continued on next page

Western and Dot Blot, Continued

Immuno-detection Procedure

This immunodetection procedure is for small membranes (60 cm^2). To blot standard size gels (~200 cm^2), scale up the required solution volumes by 3.3X.

1. Incubate the membrane in 10 ml of the appropriate Blocking Solution (previous page) in a covered, plastic dish for 30 minutes at room temperature or 4°C overnight on a rotary shaker. Decant the Blocking Solution.
2. Rinse the membrane with 20 ml water for 5 minutes, then decant. Repeat once.
3. Incubate the membrane with 10 ml Primary Antibody Solution for 1 hour, then decant.
4. Wash the membrane for 5 minutes with 20 ml of prepared Antibody Wash, then decant. Repeat 3 times.
5. Incubate the membrane in 10 ml Secondary Antibody Solution for 30 minutes, then decant.
6. Wash the membrane for 5 minutes with 20 ml Antibody Wash, then decant. Repeat 3 times.
7. Rinse the membrane with 20 ml water for 2 minutes, then decant. Repeat twice. Proceed to **Chromogenic or Chemiluminescent Detection**, below.

Chromogenic Detection

1. Incubate the membrane in 5 ml of Chromogenic Substrate until purple bands develop on the membrane. Development is complete in 1-60 minutes.
2. Rinse with 20 ml water for 2 minutes. Repeat twice.
3. Air-dry the membrane on a clean piece of filter paper or under an infrared lamp.

Chemiluminescent Detection

1. Place the membrane on a sheet of transparency plastic. Do not allow the membrane to dry out.
2. With a clean pipette, apply 2.5 ml Chemiluminescent Substrate (page 6) to the membrane surface without touching the membrane surface. Incubate for 5 minutes.
3. Blot excess Chemiluminescent Substrate solution from the membrane surface with the filter paper included in the kit. Do not allow the membrane to dry out.
4. Cover the membrane with another clean piece of transparency plastic to prepare a membrane sandwich for luminography. Expose an X-ray film to the membrane sandwich for 1 second to several minutes.

Continued on next page

Dot Blot Protocol

This protocol is used to quickly detect the presence of recombinant proteins. Be sure to spot equivalent amounts of protein for each sample.

1. Make serial dilutions of samples (purified protein, partially purified protein, or cell lysates) in 10 mM Tris-HCl, 25 mM EDTA, pH 8.0. The lowest dilution should have at least 30 ng of protein.
2. Spot 1 µl of each sample onto nitrocellulose or PVDF membrane using a dot blot apparatus.
3. Allow the membrane to air-dry.
4. Proceed to the **Immunodetection Procedure**, previous page.

ELISA

Introduction

A sample of a direct ELISA protocol using horseradish peroxidase (HRP) to detect the amount of antigen in a bacterial cell lysate is provided in this section. ELISAs can detect 1 ng/ml to 1 µg/ml antigen in a bacterial cell lysate. Sandwich ELISAs are more sensitive than direct ELISAs.

For more details on ELISA and other protocols, refer to *Antibodies* (Harlow and Lane, 1988) and *Current Protocols in Molecular Biology* (Ausubel *et al*., 1994). The table below outlines the basic steps of an ELISA.

Step	Description
1	Bind the antigen to a polystyrene, high binding microtiter plate.
2	Block the remaining sites using blocking buffer.
3	Probe the plate with an appropriate dilution of the custom antibody.
4	Incubate the plate with anti-species IgG secondary antibody conjugated to an enzyme such as alkaline phosphatase or HRP.
5	Visualize the bound protein using a colorimetric detection method.

Guidelines

Consider the items below when setting up your ELISA.

- Include controls for cross-reactivity and nonspecific binding to host cell proteins
- Be sure that all experimental conditions are kept constant to ensure reproducibility
- Determine optimal dilution of the custom antibody with your antigen
- Always include a standard curve with each plate
- Analyze samples in duplicate
- Be sure that the concentration of antigen falls within the dynamic range of the standard curve

Continued on next page

ELISA, Continued

Materials Needed

You will need the following items for ELISA:

- 96-well, high binding microtiter plate
- HRP conjugated secondary antibody
- Substrate for detection
- Phosphate buffered saline (PBS)
- Borate Buffer (0.1 M Borate buffer, pH 8.2 with 75 mM NaCl, 5 mM EDTA)
- Blocking Buffer (Borate buffer, above with 1% BSA, 1% gelatin, and 0.05% Tween 20)
- Wash Buffer (0.05% Tween 20 in borate buffer)
- Citrate Buffer (0.04 mM citrate buffer, pH 4.0)
- ABTS (2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid)
- Hydrogen peroxide

Procedure

Instructions are provided below to prepare a standard curve using a purified antigen. For analyzing lysates, see page 12.

1. Prepare a 10 µg/ml solution of antigen in Borate Buffer. This will be used for your standard curve so it should be as pure as possible.
2. Take a polystyrene, high binding, 96-well microtiter plate and serially dilute the antigen solution across the columns so that each row has a different dilution of antigen. Use 50 µl as the final volume in each well. Leave column 12 blank as a background control.
3. Cover the plates and incubate overnight at 4°C or 2 hours at room temperature for antigen binding.
4. Remove antigen solution and wash the wells with Wash Buffer three times.
5. Add 0.2 ml Blocking Buffer to each well and incubate for at least 1 hour at room temperature.
6. Remove Blocking Buffer.
7. Serially dilute the custom antibody across the rows. Use Blocking Buffer as the diluting buffer and 100 µl as the final volume in each well. Start with a 1:50 dilution in row A.

Continued on next page

ELISA, Continued

Procedure, continued

8. Cover and incubate plates at room temperature for 2 hours or 4°C overnight.
9. Remove antibody and wash wells four times with Wash Buffer.
10. For unconjugated custom antibody, add 100 µl of diluted anti-species HRP-conjugated secondary antibody to each well. Use Blocking Buffer as the dilution buffer. For the appropriate dilution of secondary antibody, see the manufacturer's instructions.
11. Incubate at room temperature for 1-2 hours.
12. Remove antibody and wash wells four times with Wash Buffer. Proceed to **ELISA Detection Reaction**, below.

ELISA Detection Reaction

The protocol uses a sensitive chromogenic substrate for detection of HRP-labeled reagents. Other detection reagents are suitable.

You will need 10 ml substrate for each 96-well plate.

1. Dissolve 5 mg of ABTS in 10 ml of 0.04 mM citrate buffer, pH 4.0.
2. Add hydrogen peroxide to a final concentration of 0.01% to the ABTS solution immediately before use.
3. Add 100 µl of the substrate solution to each well.
4. Incubate for 30 minutes at room temperature. Positives have a greenish hue.
5. Read the results at 405 nm using an ELISA plate reader.

Analysis of Experiment

Plot absorbance versus known antigen concentration on a semilog paper to analyze each antibody dilution. For a working dilution of antibody, choose the dilution that provides the maximum sensitivity over a linear range of antigen concentrations and minimum binding (< 0.05 absorbance units) for background.

Continued on next page

Analyzing Lysates

Once you have identified the optimal working dilution of the custom antibody, you are ready to analyze your lysates.

1. Prepare ~10 µg/ml solution of your lysate in Borate Buffer.
2. Take a polystyrene, high binding, 96-well microtiter plate and serially dilute the lysates across the columns so that each row has a different dilution of antigen. Use 50 µl as the final volume in each well. Leave column 12 blank as a background control.
3. Cover the plates and incubate overnight at 4°C or 2 hours at room temperature for antigen binding.
4. Remove antigen solution and wash the wells with Wash Buffer three times.
5. Add 0.2 ml Blocking Buffer to each well and incubate for at least 1 hour at room temperature.
6. Remove Blocking Buffer.
7. Add 100 µl of the appropriate dilution of custom antibody in Blocking Buffer.
8. Cover and incubate plates at room temperature for 2 hours or 4°C overnight.
9. Remove antibody and wash wells four times with Wash Buffer.
10. For unconjugated custom antibody, add 100 µl of diluted anti-species HRP-conjugated secondary antibody to each well. Use Blocking Buffer as the dilution buffer. For the appropriate dilution of secondary antibody, see the manufacturer's instructions.
11. Incubate at room temperature for 1-2 hours.
12. Remove antibody and wash wells four times with Wash Buffer. Proceed to **ELISA Detection Reaction**, previous page.

Immunoprecipitation

Introduction

The custom antibodies can be used to immunoprecipitate fusion proteins that contain the appropriate epitope. A general immunoprecipitation protocol is described below; other protocols are suitable (refer to Harlow and Lane, 1988). The table below lists the basic steps of immunoprecipitation.

Step	Description
1	Prepare a cell lysate using lysis buffers.
2	Pre-clear the lysate with Protein G Agarose slurry.
3	Incubate the pre-cleared lysate with an appropriate amount of the custom antibody and Protein G Agarose slurry.
4	Remove the supernatant and wash the resin with lysis buffer.
5	Resuspend the resin in SDS sample buffer and analyze the immunoprecipitated proteins by SDS-PAGE.

Materials Needed

You will need the following items:

- Lysis Buffer
  - RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% deoxycholate (DOC), 0.1% SDS, 50 mM Tris, pH 7.5)

  OR

  - NP-40 or Triton-X-100 lysis buffer (150 mM NaCl, 50 mM Tris, pH 8.0, 1% NP-40 or Triton-X-100)
- Protein-G Agarose slurry
- Protease inhibitor cocktail (containing 10-100 µM leupeptin, 1-10 mM EDTA, 1 µM pepstatin, and 0.2-1 mM PMSF)
- SDS sample buffer (2% SDS, 10% glycerol, 100 mM DTT, 60 mM Tris pH 6.8, and 0.001% bromophenol blue)

Continued on next page

Immunoprecipitation, Continued

Procedure

1. Lyse cells using one of the lysis buffers above or the one of your choice. For mammalian and insect cells, use approximately 5×10^6 to 1×10^7 cells/ml of lysis buffer. For *E. coli* and yeast cells, use approximately 1×10^9 cells/ml of lysis buffer.
2. Centrifuge the lysate for 20 minutes at 10,000 x g at 4°C. Transfer supernatant to a sterile microcentrifuge tube and place on ice.
3. Add 50 µl of Protein-G Agarose resin slurry (50% slurry in lysis buffer) per 1 ml of supernatant to pre-clear the lysate.
4. Rock at 4°C for 1 hour.
5. Centrifuge for 1 minute at 10,000 x g at 4°C.
6. Transfer supernatant to a sterile microcentrifuge tube and place on ice.
7. Add 2-5 µl of the custom antibody and 50 µl of the Protein-G Agarose resin slurry to the supernatant. Rock for 2-24 hours at 4°C.

   Note: You may optimize the amount of antibody used based on the initial results.
8. Centrifuge for 1 minute at 10,000 x g at 4°C. Remove supernatant.
9. Wash the resin 2X with 500 µl lysis buffer.
10. Analyze the fusion protein immune complexes using SDS-PAGE or functional assay.

    For SDS-PAGE, add 50 µl of 1X SDS sample buffer to the resin. Heat the sample at 85°C for 2 minutes. Centrifuge for 1 minute at 10,000 x g and load supernatant onto a suitable gel.

Immunostaining

Introduction

The custom antibodies can be used in immunohistochemical and immunofluorescent methods to detect fusion proteins in cells or tissues that contain the epitope.

The procedure below describes a general immunohistochemical protocol using reagents available from Invitrogen. For other protocols, refer to *Antibodies* (Harlow and Lane, 1988).

Step	Description
1	Perform deparaffinization and rehydration.
2	Unmask antigenic epitope with enzymatic digestion.
3	Block endogenous peroxidase activity with 3% H_2O_2.
4	Block non-specific binding with antibody diluent.
5	Probe the slide with an appropriate dilution of the custom antibody.
6	Incubate the slide with biotinylated secondary antibody followed by incubation with streptavidin-HRP.
7	Develop the signal using a colorimetric substrate.
8	Counter stain with hematoxylin.
9	Apply mounting medium and evaluate results with a microscope.

Materials Needed

You will need the following items for immunohistochemistry. Ordering information is on page 20.

- Auto Dewaxer
- 10X Immuno/DNA Buffer
- Pepsin
- Primary Antibody Diluent
- Universal Biotinylated Secondary Antibody
- Streptavidin Horseradish Peroxidase
- Stable Diaminobenzidine
- Auto Hematoxylin
- Universal Mount
- Ethanol
- Coplin jars and slide washer
- 3% H_2O_2 in methanol
- Oven set at 55°C

Continued on next page

Immunostaining, Continued

Guidelines

- Perform all steps at room temperature, unless another temperature is specified
- Do not allow the slides to dry during the procedure
- Perform incubation with reagents in Coplin jars

Deparaffining and Hydrating

1. Prepare 200 ml of 1X Immuno/DNA Buffer from the 10X Immuno/DNA Buffer. Label a Coplin jar, add 25 ml 1X buffer to the jar and add the remaining 175 ml to a washer.
2. To promote adherence of the tissue to the slides and to soften the paraffin, incubate the slides at 55°C for 30 minutes.
3. Immerse the slides in Auto Dewaxer for 2-5 minutes. Repeat this step twice in separate Coplin jars.
4. Immerse the slides in 100% ethanol for 10 seconds to remove the dewaxing agent and remaining paraffin. Repeat this step in a separate Coplin jar.
5. Immerse the slides in 95% ethanol for 10 seconds. Repeat this step in a separate Coplin jar.
6. Unmask the antigens on the slide by using any one of the methods described below:
   - Perform enzymatic digestion with 100 µl Pepsin at 55°C for 3 minutes **or**,
   - Incubate the slide in distilled water for 5 minutes to overnight **or**,
   - Boil the slides in 20 mM citrate buffer, pH 6.0 for 10 minutes
7. Rinse the slides with 1X Immuno/DNA Buffer from the wash bottle after incubation.

Continued on next page

Immunostaining, Continued

Detection Protocol

1. Block endogenous peroxidase activity by incubating slides in 3% H_2O_2 in methanol for 10 minutes.
2. Rinse the slides with 1X Immuno/DNA Buffer and incubate the slides in Primary Antibody Diluent for 30 minutes at room temperature.
3. Dilute the custom antibody appropriately in Primary Antibody Diluent. Add ~100 µl (or enough to cover the tissue completely) primary antibody solution onto each slide and incubate the slides at room temperature for 1 hour or 55˚C for 10 minutes.
4. Rinse the slides with 1X Immuno/DNA Buffer three times for 5 minutes each time.
5. Add ~100 µl of the biotinylated Universal Secondary Antibody onto each slide and incubate the slides at 55˚C for 4 minutes or 30 minutes at room temperature.
6. Rinse the slides with 1X Immuno/DNA Buffer as described in Step 3.
7. Add ~100 µl streptavidin HRP onto each slide and incubate the slides at room temperature for 30 minutes or at 55˚C for 4 minutes.
8. Rinse the slides with 1X Immuno/DNA Buffer.
9. To generate a colorimetric signal, add 100 µl Stable Diaminobenzidine (DAB) onto each slide, and incubate the slides at 55˚C for 5 minutes or until the desired signal intensity is reached.

Caution: DAB is a known carcinogen. Handle it with care.

10. Rinse the slides with 1X Immuno/DNA Buffer to remove excess Stable DAB.

Counter-staining and Mounting

1. Immerse the slides in Auto Hematoxylin for 30 seconds for counterstaining.
2. Rinse the slides with deionized water.
3. Immerse the slides in 1X Immuno/DNA Buffer for 1 minute.
4. Add 3 drops of Universal Mount to the tissue and apply a cover slip. To harden without a cover slip, apply Universal Mount and heat to 60˚C.
5. Evaluate the results by examining the slides with a microscope.

Appendix

Peptide Reconstitution

Introduction

After the immunization protocol is complete with the peptide, any remaining peptide is shipped with the antibodies. You may use the peptide to perform various assays with the antibodies. Prior to performing any assay, you need to choose an appropriate solvent to dissolve the peptide.

Brief instructions are included in this section to reconstitute custom peptides. For more details, refer to the Custom Peptide Storage and Dissolution manual available at www.invitrogen.com or contact Technical Service (page 21).

Dissolving Custom Peptides

When reconstitution is necessary, allow the vial to equilibrate at room temperature before opening. Remove only the material necessary and blanket the unused portion with argon or another inert gas, cap the vial, and return the vial to -20°C.

The solubility of the peptide depends on the charge and hydrophobicity of a peptide. The solvent is chosen based on the following factors:

- Nature (acidic, basic, or hydrophobic) of the peptide (see next page)
- Compatibility of the solvent with downstream applications
- Stability of the peptide in the solvent (solvent must not react or promote degradation of the peptide)

After determining the charge and hydrophobicity of the peptide, choose an appropriate solvent for the peptide (see **Water Soluble Peptides, Acidic Peptides, Basic Peptides, and Hydrophobic Peptides**, next page).

To avoid degradation, dissolve peptides in degassed, sterile, distilled water or an appropriate solvent. Once a peptide is dissolved, use within a week and store at -20°C blanketed with an inert gas. Avoid repeated freezing and thawing of peptide solutions.

Continued on next page

Peptide Reconstitution, Continued

We recommend testing a small amount of the peptide for solubility in water or various solvents prior to dissolving the entire amount of peptide.

Water Soluble Peptides

For peptides that are hydrophilic (due to the presence of >25% charged residues such as glutamic acid, aspartic acid, lysine, arginine, and histidine), dissolve peptides in distilled water. For peptides insoluble in water, see below.

Acidic Peptides

For peptides that are acidic (due to the presence of aspartic and/or glutamic acid residues), add a small amount of 5% ammonium hydroxide. Once the peptide is dissolved, you can dilute the peptide with water or aqueous buffered solution to the desired concentration. Avoid basic conditions when reconstituting peptides that contain cysteine.

Basic Peptides

For peptides that are basic (due to the presence of histidine, lysine, and/or arginine residues), add a small amount of 5% acetic acid. Once the peptide is dissolved, you can dilute the peptide with water or aqueous buffered solution to the desired concentration.

Hydrophobic Peptides

For peptides that are hydrophobic (>50% hydrophobic) due to the presence of isoleucine, leucine, phenylalanine, and/or valine residues, dissolve the peptide in minimal amount of DMSO, DMF, acetonitrile, isopropyl alcohol, or ethanol. Once the peptide is dissolved, slowly add (in drops) the peptide to a stirred aqueous buffered solution to dilute the peptide to the desired concentration. If the resulting peptide solution begins to show turbidity, the solubility limit of the peptide is reached.

Oxidation of Peptides

Peptides containing cysteine and methionine are susceptible to oxidation when exposed to air. To avoid oxidation, dissolve peptides in degassed, distilled water or an appropriate solvent.

Accessory Products

Additional Products

Additional products that may be used with the custom antibodies are available separately from Invitrogen. Ordering information is included in the table below. For more details, visit our Web site at www.invitrogen.com or contact Technical Service, next page.

Product	Quantity	Catalog no.
Nitrocellulose (0.45 µm)	20 membrane/filter papers	LC2000
Invitrolon™ PVDF (0.45 µm)	20 membrane/filter papers	LC2005
MagicMark™ XP Western Standard	250 µl	LC5602
WesternBreeze® Chromogenic Kit		
Anti-Mouse	1 kit	WB7103
Anti-Rabbit	1 kit	WB7105
Anti-Goat	1 kit	WB7107
WesternBreeze® Chemiluminescent Kit		
Anti-Mouse	1 kit	WB7104
Anti-Rabbit	1 kit	WB7106
Anti-Goat	1 kit	WB7108
Recombinant Protein G Agarose	5 ml	15920-010
Auto Dewaxer	500 ml	750112
10X Immuno/DNA Buffer	500 ml	750124
Pepsin Solution	25 ml	750102
Primary Antibody Diluent	250 ml	750104
Universal Secondary Antibody	25 ml	750122
Stable DAB	250 ml	750118
Streptavidin Horseradish Peroxidase Detection System	25 ml	750106
Auto Hematoxylin	500 ml	750107
Universal Mount	250 ml	750105

Technical Service

Contact us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).

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MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

Limited Warranty

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References

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