CAMBRIDGE RESEARCH BIOCHEMICALS

ANTIBODY VALIDATION

Cambridge Research Biochemicals offers a fully flexible custom Western Blot analysis service to complement its custom generated Target Antibodies™.

Western Blot validation may be carried out on any CRB generated custom polyclonal or monoclonal antibody.

Target Antibodies™

- Antibodies can be raised to haptens, peptides and proteins
- Free consultation and peptide antigen design utilised for post-translation modification sites such as phosphorylation, glycosylation, sulphation and methylation
- Isoform, neo-epitope and splice site specificity targeted via peptide antigens and tactical purification strategies
- Choice of multiple species available for conserved regions or multiplexed assays

Fit for Purpose

Immunoblotting (Western Blot analysis) can be used to determine important characteristics of protein antigens in an extract of cells or tissues and can be used to validate the primary antibody. CRB can determine the usefulness of custom derived antibodies by performing first pass Western Blot analysis to provide the antibody user with the following valuable information:

- Confirmation of anti-protein response for peptide derived antibodies
- Screening of a variety of controls and customer samples
- Validation of anti-peptide antibodies using peptide blockade specificity controls
- Validation of antibody efficacy against post translational modification sites
- Detailed methodological report provided including working concentrations for primary and secondary antibodies
- Digitised images supplied using high-sensitivity detection system

Customer Support

- Tailored to specific customer validation requirements
- Pre-screening of animal sera against target prior to immunisation
- Screening of anti-peptide sera to assess anti-protein target response prior to affinity purification
- Protocol optimisation against target using negative and positive controls

ANTIBODY VALIDATION

Step 1

Sample preparation

- Samples received (cell lysates/ protein or tissue)
- Samples prepared and the 3-dimensional structure denatured

Step 2

Running Sodium Dodecyl Sulphate Polyacrylamide (SDS-PAGE) gel

- 20-30µg of total protein/ cell lysates/ tissue homogenates or 10-100ng of purified protein loaded into the lanes of an SDS PAGE gel
- Gel ran using Mini-Protean Tetra Cell

Step 3

Transferring protein to nitrocellulose membrane

- Samples transferred from SDS-PAGE gel on to a nitrocellulose membrane

Step 4

Antibody labelling

- Nitrocellulose membrane is blocked followed by incubation with the 1º antibody
- Membrane is washed, followed by incubation with HRP linked 2º antibody, followed by a series of washes

Step 5

Detection

- Digitised images are acquired using a high sensitivity detection system

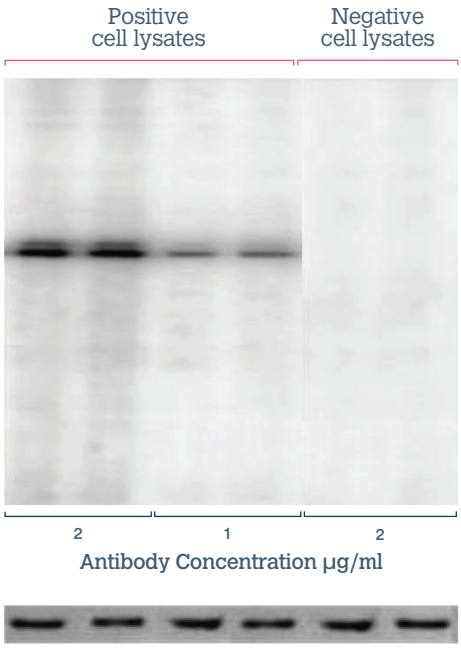
Step 6

Analysis

- Specificity controls assessed
- Molecular weight of bands determined
- Report to customer

Western Blot

Phosphorylated specific antibody



- Standard to show sample loading control
- Samples are evenly distributed

Pre-screening sera

Target detection
Test bleeds

Crude sera analysis
Purification decision

Antibody optimisation

Competition binding

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