Antigen – peptide
Covers a pool of various epitopes
- 7-30 amino acids
- long peptide, carrier conjugated (K/H, BSA)
- Thiol group of cysteine
- Peptide will target C or N terminus; should not be located in part of a protein which is going to be processed, as well as in domains repeated in other proteins
- Internal cysteines should be avoided since epitopes around this amino acid are going to be lost
- Consider if a peptide should target one specific isoform or can be repeated in all isoforms from a given protein family
- A peptide mix can also be used for immunization as it will cover more epitopes
- Immunization with a peptide without a carrier is possible for longer peptides (>30 aa)

Antigen – protein
Covers a pool of various epitopes
- Recombinant protein
  - Can be either tagged or cleaved
  - Protein buffer composition should not be harmful to animals
  - Ideal protein concentration: 1 mg/ml
  - For protein purity issues, the protein can be excised from a gel
  - Required amount for immunization of two animals is 1 mg or less
- Native protein purified from endogenous source
  - Risk for one or more isoforms present in such preparation

What an antibody looks like
- Variable light chain
  - Can bind to your protein occurs through antigen binding domain (epitope)
- Constant light chain
- Variable heavy chain
- Constant heavy chain

Linear Epitope
Recognized in WB, IHC (denatured)
- Antibody
- Protein

Discontinuous Epitope
Recognized in IP, WB, ChIP (native)
- Antibody
- Protein

Antibodies and Good Laboratory Practice
- Store antibodies in 4°C/-20°C (short-term) or -20°C/-80°C (long-term)
- Store chicken antibodies (IgY) in 4°C with preservation of bacterial growth
- Prepare aliquots
- Primary and secondary antibodies should not be re-used
- Affinity purification is recommended for techniques like ChIP, IP, IHC
- Buffers never be done on whole material at once
- Optimize used protocols. Each antibody-antigen interaction is unique

Tips and Tricks for Antibody Production and Validation Process
- How to Obtain Good Results

Antigen
- Why is pre-immune serum screening important?
- What an antibody looks like
- Choice of host species

Animal
- Why is pre-immune serum screening important?
- What an antibody looks like
- Choice of host species

Analysis
- Before immunization
  - Sample extraction
    - Fast and efficient
    - Bead beater, sonication or mortar and pestle: the chosen method needs to be optimized for a given sample, to allow its prompt and efficient processing.
    - Extraction buffer containing LPS, HEPEs, or TRIS: analysis of freshly extracted samples and optimization of the extraction buffer are highly recommended. For some proteins HEPEs may provide much better results.
  - Concentrate your target protein
    - Make sure your protein samples are of best quality as no target protein on a membrane means no antibody detection.

Antibody validation check list
- Direct approach
  - Reliable knockdown/knockout mutant
  - Immunoprecipitation and MS of its product confirms protein identity (IP-MS)
- Indirect approach
  - Antibody is recognizing recombinant protein
  - Band of the same MW as the target protein is present in pre-immune
  - Peptide neutralization
  - Detection of protein at the correct MW (processing sites and modifications can affect apparent MW)
  - Low cross-reactivity
  - Samples from specific conditions, up or down regulating expression of target protein, are used
  - Same band is recognized by independent antibodies to the same target

Membrane type
- Does it matter?
  - Various membranes have different protein retention. PVDF membranes can be dried in air for a few hours to increase protein binding. In case of increased background signal, a change of membrane type is recommended.

Testing - variability in obtained results
- Why more than one animal should be immunized

Primary antibody incubation time
- Standard Western blot protocol
- Rapid Western blot protocol with Agrisera Incubation

Blocking reagent comparison
- 5% BSA/NRt
- 5% non-fat milk/0NT

Membrane transfer
- Wet (tank) transfer
- Dry transfer
- Semi-dry transfer
- PVDF
- Immobilon-P
- XCell® gel transfer system (BioRad)
- PVDF transfer can be also used for immunization as it will cover more epitopes

Protocol for unknown / not working antibody
- Check sequence used to elicit the antibody you are working with. Is it conserved in your target protein? For catalogue antibodies, contact the supplier
- Include positive and negative controls for Western blot procedure
- Use cellular fraction/organ in which the protein of interest is expressed
- Include pos and neg controls for Western blot procedure
- Lower MEOH (20%)
- Increase SSS
- Remove buffer
- Sensitivity detection: chemiluminescence or fluorescence is preferred over chromogenic

If your antibody DOES NOT WORK
- Contact Agrisera support
- Live chat www.agrisera.com
- E-mail: support@agrisera.com
- Phone: +46 935 33 000
- Or use Agrisera’s Western blot resources www.agrisera.com/western-blot
- www.agrisera.com/rapid-wb
- www.agrisera.com/igy
- www.agrisera.com/igg
- www.agrisera.com/loadingcontrols
- www.agrisera.com/peptide-synthesis
- www.agrisera.com/incubationcontrols
- www.agrisera.com/primary-antibody-concentration
- www.agrisera.com/functional-blot
- www.agrisera.com/functional-blot-rapid
- www.agrisera.com/functional-blot-igy
- www.agrisera.com/functional-blot-igg
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