Tips and tricks for antibody production and validation process - how to obtain good results

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Three crucial components of successful antibody production

Antigen

- Peptide-Protein Conjugate
  - 7 – 30 amino acids long peptide, carrier conjugated (KLH, BSA)
  - by Thiol group of cysteine
  - is targeted C or N-terminal, should not be located in parts which are processed, should not be located in domains repeated in other proteins
  - Avoid internal cysteine (peptide around this amino acid will be lost)
  - Should your peptide be isoform-specific or repeated in many (all) isoforms of investigated protein family?
  - Peptide mix (2 peptides) can be also used, covers more epitopes
  - Immune product
  - Antibody affinity purified

Host

- Recombinant protein
  - Purified or cloned
  - Buffer composition should not be harmful for animals
  - protein concentration ~1 mg/ml
  - purity issues (gel page ok)
  - 1 rabbit, 5 guinea pigs or less
  - Native proteins purified from tissue
  - Risk for one or many isoforms in obtained preparation

Testing

- Sample extraction = Fast & efficient
  - bead beater extraction vs. mortar and pestle
  - Non-protein blocker: 0.1-1 % PVP40, 1h/RT

Choice of host species

Polyclonal antibody

- Will recognize a pool of several epitopes
  - Amount of produced antibody: 0.1-1 mg/ml

Monoclonal antibody

- Will recognize a single specific epitope
  - 2-5 amino acids
  - Will recognize a single specific epitope
  - Peptide-Protein Conjugate

Before immunization: Pre immune serum screening why is it important?

Even before immunization some animals can develop antibodies which give a signal in proximity of our target protein. Antibody screening is vital to know if your animal has pre immune serum.

What an antibody looks like

- Variable Heavy chain
  - Binding to your protein occurs through antigen binding domain (epitope)
- Constant Light chain
- Constant Heavy chain

Antibodies and good laboratory practice

- Short (21°C) and long term storage (4°C), chicken antibodies in 4°C, aliquots
- Re-using antibody primary or secondary antibody is not recommended for a quantitative western blot
- Affinity purification (never done for a whole material at once)
- Protocol optimization

Antigen – peptide

(covers smaller epitope pool)

- Peptide-Protein Conjugate
- 7 – 30 amino acids long peptide, carrier conjugated (KLH, BSA)
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- Immune product
- Antibody affinity purified

Recombinant protein

(covers a pool of various epitopes)

Polyclonal antibody

- Will recognize a pool of several epitopes
  - Amount of produced antibody: 0.1-1 mg/ml

Monoclonal antibody

- Will recognize a single specific epitope
  - 2-5 amino acids

Every step of Western blot is important for data quality

Consider MW of your target protein

Polyclonal antibody

- Recognized in IP, WB
- Discontinuous Epitope
- Example: fractionation
  - Requires shorter transfer time
  - Easily migrates from a gel
  - Reliable mutants are used
  - Detected protein has correct MW

Monoclonal antibody

- Recognized in IP, WB
- Continuous Epitope
- Example: epitope enrichment
  - Detects protein in SDS-PAGE
  - Does not require longer time to become visualized

Sample extraction = Fast & efficient

- Bead beater extraction vs. mortar and pestle
- Non-protein blocker: 0.1-1 % PVP40, 1h/RT
- Blocking, primary and secondary antibody incubations
- Include positive and negative controls for Western blot procedure
- Further reading:
  - Agrisera IncuBlocker
  - Pre immune serum screening why is it important?