Monoclonal Antibody Generated by DNA Immunization: Powerful Tools for Antibody Drug Development

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2. What is DNA Immunization?
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How are Monoclonal Antibodies Made?

1. Mouse challenged with antigen
2. Spleen Cells
3. Myeloma Cells
4. Fusion
5. Hybridomas
6. Culture in HAT Medium
   - Select for positive cells
7. Harvest monoclonal antibodies
GenScript Multiple Immunogen Designs

- **Peptides** designed by OptimumAntigenTM design tool
- **Soluble protein** or excellular domain (ECD) recombinant protein production
- **Whole cell** immunization: GenScript develops stable cell line/transient cells for immunization and screening
- **Genetic (DNA)** immunization to deliver the target DNA plasmid into the host animals by Gene Gun technique (today’s topic)
- **Virus like particle (VLP)** that contains enriched target protein
Why MSM-Antibodies are Difficult to Generate?

MSM: Multi-Spanning Membrane Proteins
- Small, constrained, post-translation modified extracellular loops;
- Multi-domain epitopes may be required;
- Native state is required (antibodies against peptides or unfolded proteins fail to recognize native antigen);
- Multiple difficulties in expression, purification, and maintaining the native state.

Extracellular regions available for binding
- >90% Single spanner
- 25-30% GCR
- 17% 7TM
- 24% 24TM

Make Research Easy
DNA Immunization for Antibody Generation

1. Target cDNA
2. Cloning
3. Immunization
4. Immune Response
5. Fusion & Screening
6. Monoclonal Antibody Development

- Target cDNA
- Cloning
- Immunization
- Immune Response
- Fusion & Screening
- Monoclonal Antibody Development

Make Research Easy
## DNA Immunization Features

### Applications and Advantages of DNA Immunization Service

<table>
<thead>
<tr>
<th>Key Advantages</th>
<th>Antibody Development for Membrane Proteins and Problematic Antigens</th>
<th>Early DNA Vaccine Development</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Streamlines Ab production against membrane proteins and other problematic antigens</td>
<td>• Superior <em>codon optimized gene synthesis</em> technology ensures quality antigen production in vivo</td>
</tr>
<tr>
<td></td>
<td>• Eliminates need to produce and purify target protein <em>in vitro</em></td>
<td>• Optimized plasmid vectors and immunization protocols promote transfection efficiency</td>
</tr>
<tr>
<td></td>
<td>• Abs produced recognize native protein structure</td>
<td>• Specialized adjuvant and immune mediators substantially enhance immune response</td>
</tr>
<tr>
<td></td>
<td>• Protein is produced in small quantities <em>in vivo</em> driving production of high affinity Abs</td>
<td>Flexible customization options available</td>
</tr>
</tbody>
</table>

Flexible customization options available
Readily integrated downstream applications for antibody drug development
Advantages

- **Shortened Development and Production Time**
  - Synthesis and purification of proteins no longer necessary
  - Animals can be immunized in as little as 3 days
  - DNA immunization induces a qualitatively superior response in that Abs can be induced after a single vaccination with DNA

- **Affinity and Specificity**
  - Protein is produced in very small quantities, thus it is more likely to drive production of high-affinity Abs
  - Slow, consistent presentation to immune system favors production of high-affinity Abs
  - Enhances quality of Abs produced since those that recognize native folded protein are most useful in proteomics
  - Molecular chaperones are available to help fold proteins that are normally difficult and misfolded proteins are eliminated via the normal proteasome pathway.
  - DNA can be made in highly pure form, reducing the likelihood of generating Abs to contaminants

- **Can circumvent technical issues associated with problematic antigens**
  - Viable method to generate Abs against transmembrane proteins (i.e. GPCRs and ion channels), large protein domains, insoluble proteins, toxic proteins, proteins containing disulfide bonds, and PTM-modified proteins
## Applications

### Solution for Membrane Protein and Problematic Antigens

- Streamlines Ab production against membrane proteins and other problematic antigens
- Eliminates need to produce and purify target protein
- Abs produced recognize native protein structure
- Protein is produced in small quantities *in vivo* driving production of high affinity Abs

### Early DNA Vaccine Development

- Superior codon optimized gene synthesis technology ensures quality antigen production *in vivo*
- Optimized plasmid vectors and immunization protocols promote transfection efficiency
- Specialized adjuvant and immune mediators substantially enhance immune response

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**Flexible customization options available**

**Readily integrated downstream applications for antibody drug development**

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**Diagram:**
- You provide the DNA sequence
- GenScript DNA Immunization
- Protein antigen expression *in vivo*
- Custom antibody production
DNA Immunization: A Powerful Solution for...

◆ **Individuals involved in:**
  - Ab drug development
  - Early DNA vaccine development

◆ **Individuals with problematic antigens:**
  - Transmembrane proteins (i.e. GPCRs and ion channels)
  - Large protein domains
  - Insoluble proteins
  - Toxic proteins
  - Proteins containing disulfide bonds
  - PTM-modified proteins
  - Unstable proteins
  - Insufficient amount of protein production *in vitro*
Gene Immunization: DNA design

**DNA Codon Optimization**

- Codon usage bias
- GC content
- Negative CpG islands
- RNA instability motif (ARE)
- Repeat sequences

**Expression Vector Selection and Functional Element**

- pcDNA3.1 or pCAGGS-plasmid construction
- His/Flag Tag-expression identification
- KOZAK Sequence-Increase protein expression
- ImmunoPlus Sequence-overcome immune tolerance

![Plasmid construction diagram]
DNA Immunization

• Gene Gun to deliver plasmid DNA
• Immunization target tissue
  - abdominal skin and muscle cell
• DNA immunization + protein/cell/membrane boost
• Pressure of helium
• Optimized Adjuvant

<table>
<thead>
<tr>
<th>Date</th>
<th>Immunization and bleed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>Bleed 0.2 ml (yields 0.1 ml pre-immune serum)</td>
</tr>
<tr>
<td></td>
<td>1st immunization</td>
</tr>
<tr>
<td>14th day</td>
<td>2nd immunization</td>
</tr>
<tr>
<td>21st day</td>
<td>Test bleed by FACS or ICC</td>
</tr>
<tr>
<td>28th day</td>
<td>3rd immunization</td>
</tr>
<tr>
<td>35th day</td>
<td>Test bleed by FACS or ICC</td>
</tr>
<tr>
<td>42nd day</td>
<td>4th immunization</td>
</tr>
<tr>
<td>49th day</td>
<td>Test bleed by FACS or ICC</td>
</tr>
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</table>
DNA Immunization Flowchart

Condron optimization and DNA synthesis

Plasmid expression identification

Host immunization with gene gun

Serum tested by FACS

Cell fusion and culture

Positive clones screened by FACS

FACS assay

ICC assay

WB assay

ELISA assay
### Comparison of Immunogen Preparations

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Native Conformation</th>
<th>Immunogen Concentration</th>
<th>Immunogen Purity</th>
<th>Success Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified Recombinant Protein/ECD</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>None</td>
</tr>
<tr>
<td>Reconstituted Protein in Vesicles/Membrane</td>
<td>+/-</td>
<td>+++</td>
<td>+++</td>
<td>Low</td>
</tr>
<tr>
<td>Peptides</td>
<td>+/-</td>
<td>+++</td>
<td>+++</td>
<td>Low</td>
</tr>
<tr>
<td>Over-expressing cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Yes</td>
</tr>
<tr>
<td>DNA</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>Yes</td>
</tr>
<tr>
<td>Lipoparticles</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Approaches used for generation of antibodies against MSM
Advantages of the GenScript DNA Immunization Service

**Advantages in Gene (DNA) immunization:**

1. Benefit for evoking immune response on **natural** epitopes, e.g. GPCR
2. Bypassing protein preparation steps, time saving and cost effective

**GenScript Experience:**

1. Optimized plasmid vectors, codon optimization, and immunization protocol to promote transfection efficiency
2. Special adjuvant and immune mediators
3. Gene gun to deliver DNA cartridge
4. Successfully delivered cases (finish >20 projects, like GLP1R)
5. Our experienced team includes renowned expert and GenScript consultant, Dr. Shan Lu, a pioneer in the field of DNA vaccination.

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**Titer test after boost immunization**

![Graph showing titer test results over boost immunization stages](image)
Case Study (1)

Anti-GLP1R mAbs generated by gene immunization

<table>
<thead>
<tr>
<th>10F7E3</th>
<th>5F11F9</th>
<th>1F1E7</th>
<th>Mouse IgG control</th>
</tr>
</thead>
</table>

Flow cytometric analysis of CHO-K1/GLP1/Gα15 stable cells expressing GLP1R (GenScript, M00451) and CHO negative control cells with three mouse anti GLP1R monoclonal antibodies (red and black respectively). The signal was developed with iFluor647 conjugated Goat Anti-Mouse IgG.
Immunocytochemistry/Immunofluorescence analysis of HEK293 cell transfected with GLP1R plasmid (a) and non-transfected HEK293 cells (b) using mouse anti GLP1R (1F1E7) monoclonal antibody (4 µg/ml). The signal was developed with iFluor488 conjugated Goat Anti-Mouse IgG.
Indirect ELISA analysis of Virus like particle (VLP) expressing GLP1R by mouse anti GLP1R (1F1E7) monoclonal antibody prepared by DNA immunization.
Western blot analysis of Virus like particle expressing GLP1R with mouse anti GLP1R (1F1E7) monoclonal antibody.

Lane 1. GLP1R Antibody, mAb, Mouse (1 µg/ml)
Lane 2. Mouse IgG control (1 µg/ml)

Predicted size: 57 kDa
Observed size: 57 kDa

The signal was developed with IRDyeTM800 Conjugated affinity Purified Goat Anti-Mouse IgG.
## DNA Immunization Protocol Details* (SC1693)

<table>
<thead>
<tr>
<th>Step</th>
<th>Specification</th>
<th>Timeline</th>
</tr>
</thead>
</table>
| Gene Synthesis & Validation   | • Codon optimization  
• Gene synthesis & plasmid preparation  
• In vitro cell transfection for expression validation | 2-3 weeks  |
| DNA Immunization              | • DNA immunization via gold particle bombardment with gene gun  
• Test bleed by ELISA | 6-10 weeks |
| Cell Fusion & Screening       | • Animals selected for fusion based on titer  
• Primary screening by whole cell based ELISA  
• Customer can evaluate hybridoma supernatants and select the top clones for their application  
• Additional screening options available | 4-6 weeks  |
| Subcloning, Expansion & cryopreservation | • Hybridomas are subcloned by limiting dilution according to the evaluation result from the customer, then expanded & frozen | Based on project |
| Monoclonal Antibody Production (optional) | • Production of mAbs for each cell line with roller bottle culture  
• Purification  
• ELISA results | Based on project |

GenScript’s DNA immunization service has been designed to seamlessly combine with several of our popular custom Ab services including:

- **Anti-ID Ab development services** – Powerful tools for antibody drug PK/PD and immunogenicity studies
- **Custom mAb services** – Fully customizable mAb development packages tailored to meet your specific needs
- **MamPower™ recombinant mAb services** – Guaranteed production service provides 50 mg purified Ab
- **Ab scale-up services** – High throughput, large-scale Ab production suitable for industrial-size yields

### Quotation and Ordering

[Get a Quote Now](#)

*Via email, phone, or fax*
Sequence to purified antibody service with no need to provide an antigen
Advantages of GenScript Custom Abs

◆ Sequence to purified antibody service with no need to provide an antigen
◆ Optimized immunization using our OptimumAntigen™ design tool and intelligent Antigen Strategy increasing specificity and affinity of antibodies
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◆ Guaranteed results: quantity of antibodies or hybridoma, ELISA titer, and WB guarantee (varies with specific package)
Advantages of GenScript Custom Abs

◆ Sequence to purified antibody service with no need to provide an antigen
◆ Optimized immunization using our OptimumAntigen™ design tool and intelligent Antigen Strategy increasing specificity and affinity of antibodies
◆ Guaranteed results: quantity of antibodies or hybridoma, ELISA titer, and WB guarantee (varies with specific package)
◆ Fast turnaround time: delivery of purified pAb or development of specific hybridoma in 45 days.
◆ Certified facility: AAALAC International accreditation and OLAW certification, demonstrating our commitment to responsible animal care and use.
Variety of GenScript Antibody Services

- **Polyclonal Antibody Services**
  FAST pAb Services-PolyExpress™, Standard pAb Services

- **Monoclonal Antibody Services**
  FAST mAb Services-MonoExpress™, Custom mAb Services, Premium Hybridoma Services

- **Phospho-Specific Antibody Services**
  Phospho-Specific pAb and mAb Services

- **Scale-up Antibody Services**
  Scale-up pAb and mAb Services, *In vivo* Ascite production, *In vitro* Roller bottle production

- **Specialized Antibody Services**
  Antibody Drug Development, Immunoassay, Purification, Modifications, Conjugation
Methylation protects microRNAs from an AGO1-associated activity that uridylates 5’ RNA fragments generated by AGO1 cleavage.
Yu B, Chen X, Vinovskis C, etc.
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Hyper-sensitive to high light1 Interacts with low quantum yield of photosystem II1 and functions in protection of photosystem II from photodamage in Arabidopsis.
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Hosmani PS, Kamiya T, Danku J, etc.
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PfSETvs methylation of histone H3K36 represses virulence genes in Plasmodium falciparum
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http://www.genscript.com
Thank you for your participation

We wish you all success in your research

Email me: Dawei.Sun@GenScript.com

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April 16, 2015/2:00 pm EST

Testing chemotherapy related cognitive dysfunction in animals with GenScript – Amy Mendenhall, PH.D.

April 30, 2015/2:00 pm EST

Clone less, know more: efficient expression optimization of proteins and pathways using the RBS calculator – Prof. Howard Salis, Penn State University
References

