Converting rabbit hybridoma into recombinant antibodies with effective transient production in an optimized human expression system

Presented at HAH Belfast 2016 by

Dr. Tim Welsink
Molecular Biology
Transient Gene Expression

HAH Belfast 2016
RAB-MAB CONVERSION

monoclonal rabbit hybridoma AB

recombinant rabbit AB produced by TGE

1) cDNA sequencing
2) Vector design, preparation
3) Transient production
4) Purification
5) QC

Hybridoma rab. AB yield

mg rab IgG total

1L spinner
30L BR batch
2L BR run 1
2L BR run 2
Sequence alignment HC variable region:

Complementarity Determining Regions (CDRs) are colored, mismatches are highlighted

Primer sequences from
Seeber et al., 2014 & Rader et al., 2000
Sequence alignment LC variable region:

Complementarity Determining Regions (CDRs) are colored, mismatches are highlighted

Primer sequences from
Seeber et al., 2014 & Rader et al., 2000
CASE STUDY

- cDNA Design
- IgG gene synthesis
- Cloning into expression vector
- Low-endotoxin plasmid preparation
- Transient gene expression
CASE STUDY

- Transient gene expression
  - Cell line: HEK or HEK-INV
  - Transfection protocol: optimized
  - Culture protocol: optimized
  - Scale: 50 to 1,500 ml
  - Production for 7 days
  - Harvest by centrifugation

- Purification
  - AF-rProtein A-650F (Tosoh Bioscience)
  - Äkta chromatography system
  - Elution by low pH
  - Dialysis to PBS pH 7.4
RESULTS
- 4 Batches produced
- Different production scale
- 2 cell lines

![Graph showing titers in production scale, cell line comparison between HEK and HEK-INV.](image)
CASE STUDY

- QC: capillary gel electrophoresis for purity

**Reducing conditions**, Agilent 2100-BioAnalyzer, Protein 230 Kit

Purity ≥ 98%

**Non-reducing conditions**, Agilent 2100-BioAnalyzer, Protein 230 Kit

Purity ≥ 85%
CASE STUDY

● Analytical SEC for determination of aggregates

![Graph of molecular weights and protein detection](image)

- **AppliChrom® ABOA-ProteSep S-L 5µ, 300mm x 8mm**
CASE STUDY

Recombinant rab-mab

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Hybridoma rabbit AB

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Right: CGE
Below: analyt. SEC
Summary

- We had to address various needs to aim the goal
- Improvement of established methods
- New methods developed
  - Sequencing of Fv, production platform
- Case study rab-mab conversion & production
- Production and purification of 4 independent batches

Outstanding:
- Detailed results upon activity of the recombinant vs. hybridoma rab-mab

Conclusion:

New rab-mab production system allows to generate mg to g scales recombinant rabbit IgGs by transient gene expression within weeks.
ACKNOWLEDGEMENTS

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Vanessa Vater

Dr. Sabrina Schindler
Mohamad Eidi

Molecular Biology Lab
Downstream Processing Lab
QUESTIONS

Please leave your question in our linkedin group transient-transfection or write use our contact form