

# Rationale for Antibody Expression

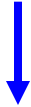


- Rapid supply of pure Ab for in vitro characterizations
- Cost-effective procurement of Ab variants for antibody optimization/screening and lead candidate identification
- Obtain  $\geq 1$  g of pure Ab to support in vivo studies for early Proof of Principle
- Predict/anticipate development issues related to expression, purification, stability and formulation of therapeutic Ab

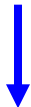
# Step 1: DNA Synthesis & Cloning



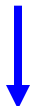
AA sequences provided by Clients



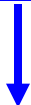
Codon Optimization



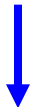
DNA Synthesis



V<sub>H</sub> and V<sub>L</sub> cloning into a single vector



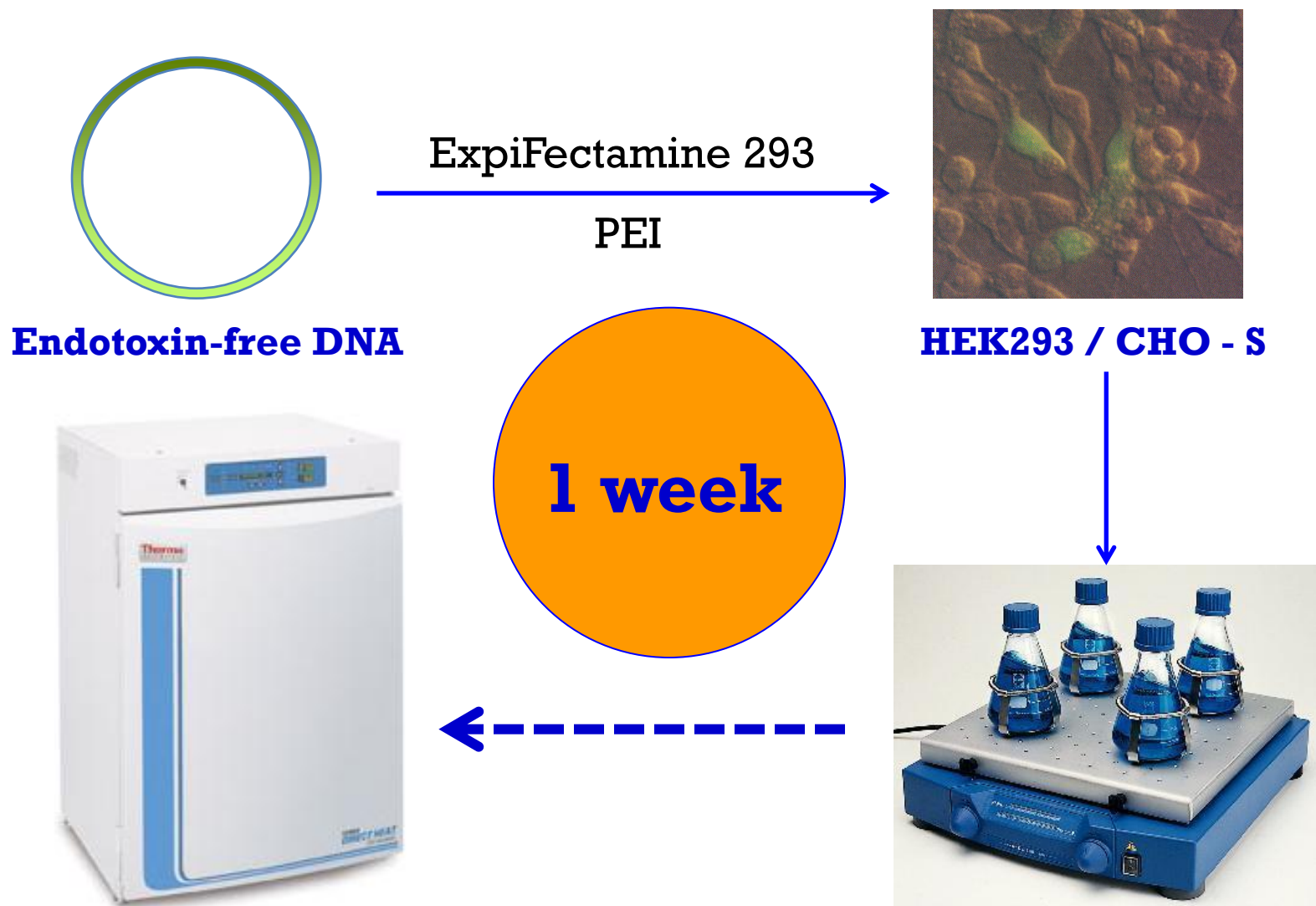
Clone validation by sequencing



Endotoxin-free large DNA prep

**2 weeks**

## Step 2: Transient Transfection



# Step 3: Purification



Clarify supernatant

**1 week**



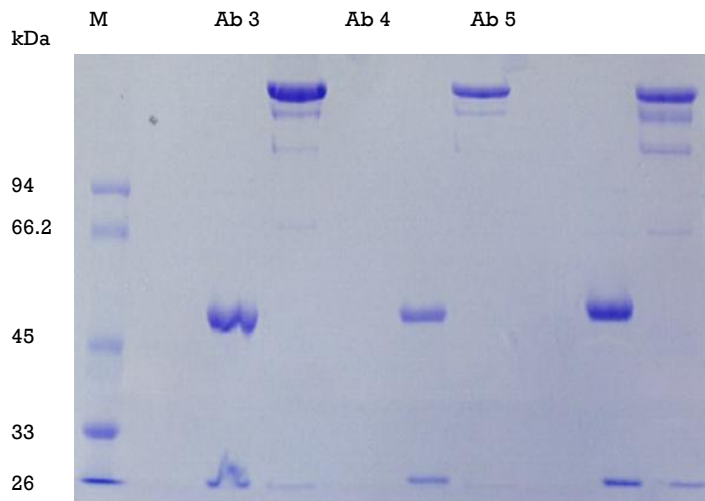
- Protein A Resin
- Protein L Resin
- Kappa-Select
- Ni-NTA Agarose
- HisTrap HP
- Superdex 200
- Superdex 75
- HiTrap SP HP
- HiTrap Q HP
- HiTrap Capto S
- HiTrap Phenyl HP

**Purified Ab  
Ready for QC**

Sepharose G-25  
Buffer exchange

**1 - 3  
Purification  
Steps**

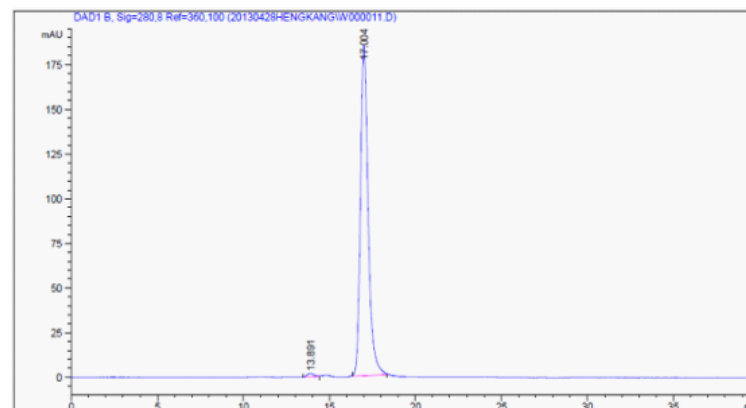
## Step 4: Rigorous QC



**SDS-PAGE**

Antibody	Endotoxin (EU/mg)	260:280 Ratio
1	0.014	0.52
2	0.015	0.56
3	0.014	0.53
4	0.014	0.56
5	0.013	0.55

**Endotoxin level  
A260:280**



**SEC-HPLC  
Detection @ 280 nm**

Plus any additional  
tests that are  
specific to your  
needs

**Completed in 2 Days**

# Custom Service List (Capability)



- Antibody/Fab expression in mammalian cells
- Recombinant protein (Fc fusion/His tag) expression in *E. coli*, insect cells, and mammalian cells
- Hybridoma gene cloning and sequencing
- Ab humanization/half life extension
- Endotoxin-free large DNA prep
- Stable cell line construction
- DNA cloning
- VLP production

- Two molecular biology labs for cloning/DNA prep
- Two tissue culture rooms for growing mammalian cells and purifying antibodies
- 10 sets of Thermo CO<sub>2</sub> incubators equipped with IKA KS 260 shakers
- One 30 L fermenter

**We can handle > 30 projects a month!**

# Summary: Our Advantages



- **High expression titer**

- A better chance to express difficult-to-express monoclonal Ab or protein, thus avoiding complications in using multiple vendors
- Reduce the workload in purification

- **Shortened timeline**

- Gene synthesis 1 week
- Cloning & DNA prep 1 week
- Expression, purification, and QC 2 - 3 weeks

**Total time ~ 5 weeks**

- **Competitive price**

- **Amenable to high throughput Ab production**