# Production of proteins for medical use in TG silkworms

## ➢ Development of animal therapeutic drugs

- Collaborative development of influenza vaccines with Institute of Biological Resource
- Collaborative development of animal drugs with ZENOAQ

## ➢ Development of human therapeutic drugs

- Advantage confirmation of TG silkworm system

  Cost advantage: Cost less than mammalian cell cultures

  Carbohydrate structure:

  More closely resembles human-type than insect-type
  (Addition of GlcNAc at non-reducing ends, Coreα1, 3-fucose free)

  Coreα1, 6-fucose free (Possibility of antibody production with high ADCC activity)

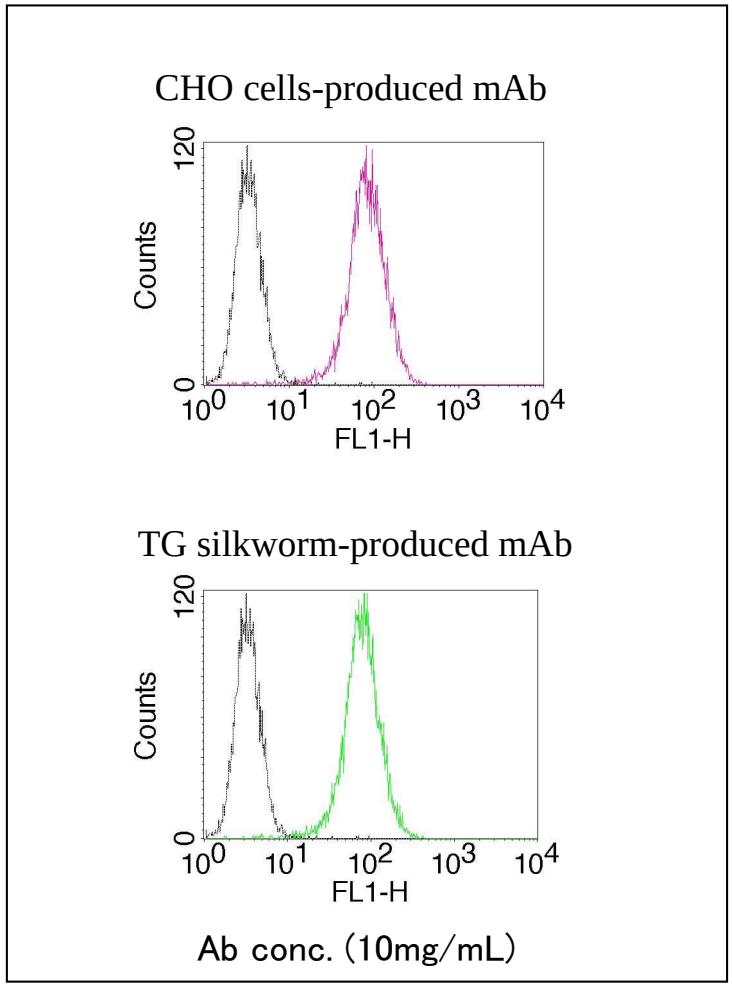
- Selection of candidate proteins

  Antibody drugs (Confirmation of behavior and low cost production)

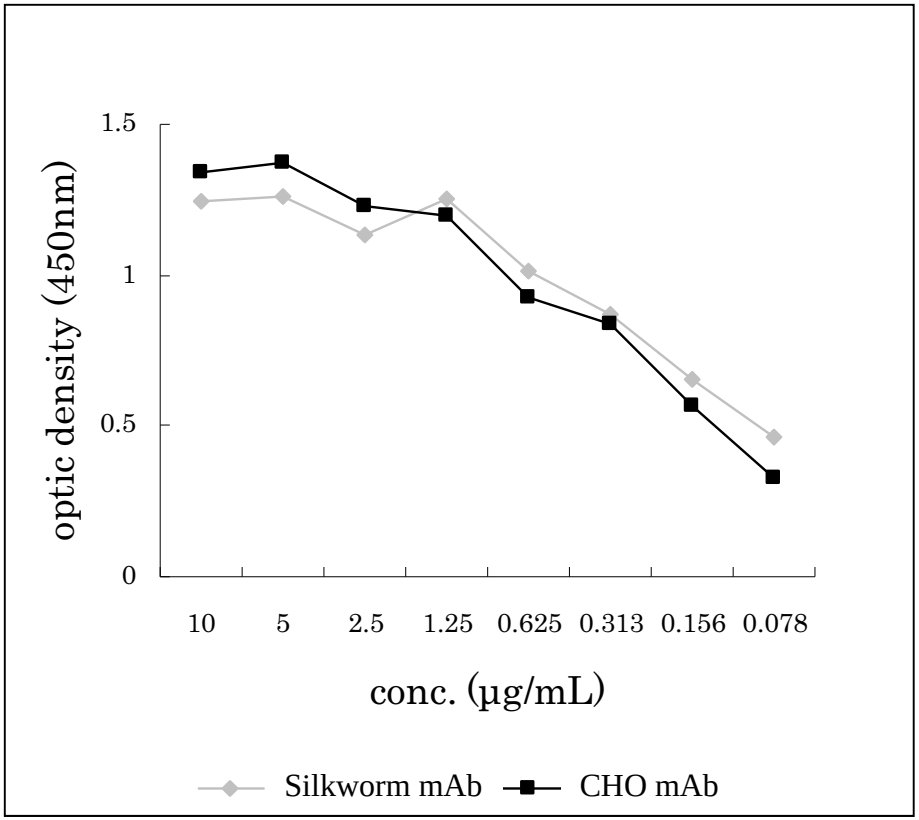
  Fibrinogen (Confirmation of coagulation behavior and low cost production)

# Evaluation of a humanized antibody produced by TG silkworms

## Evaluation by FACS

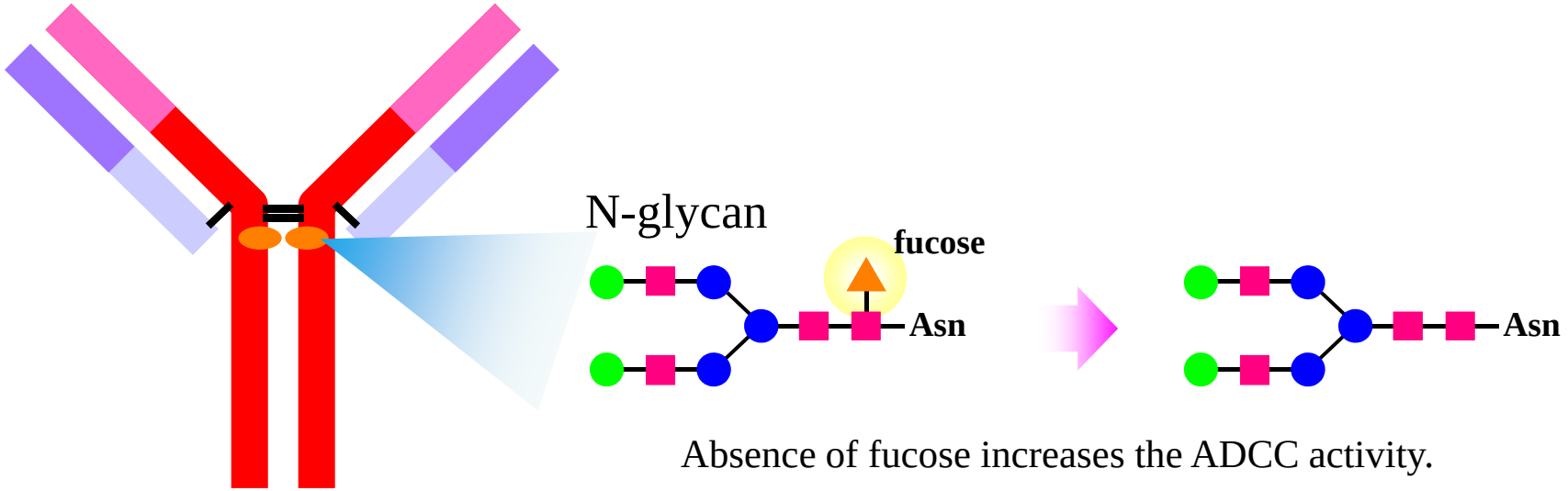



## Evaluation by cell ELISA




**A humanized mAb produced by silkworms exhibited similar behavior to those produced by CHO cells.**

# Absence of fucose in the N-glycan of the TG silkworm-produced antibodies



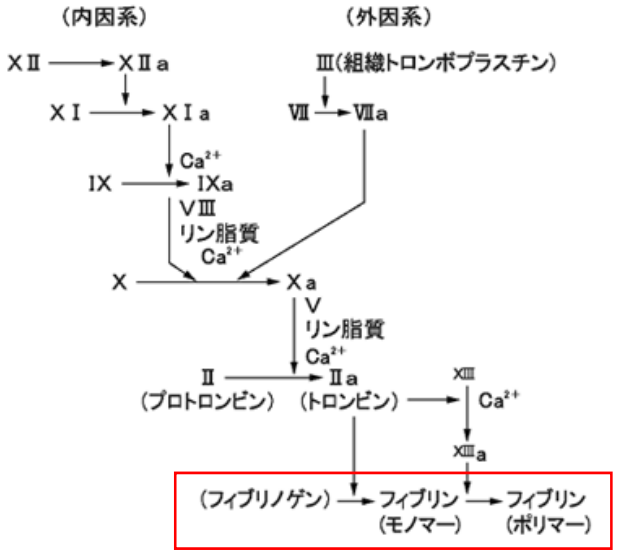

Absence of fucose increases the ADCC activity.

**The N-glycan in TG silkworm-produced antibody does not contain fucose.**

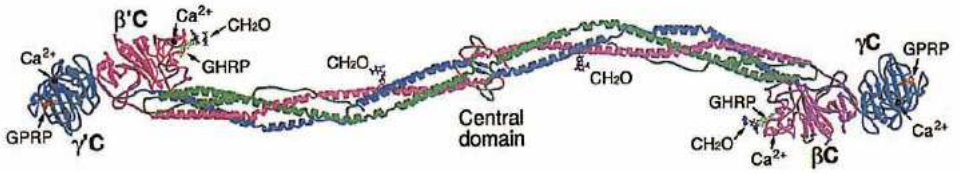
**The TG silkworm system might be beneficial for the production of antibody drugs whose main action mechanism is ADCC activity.**

# Fibrinogen

- One of blood coagulation factors directly involved in hemostasis. It clots in a glue-like form.
- Large protein with a molecular mass of 340 kDa consisting of Aα-, Bβ-, and γ-chains.
- Used as a therapeutic agent to treat congenital hypofibrinogenemia.
- Many patients have been infected with hepatitis viruses through fibrinogen products that used virus-inactivated human blood as a source of fibrinogen.
- Recombinant expression systems are sought, but no system for the commercial production on a large scale has been developed.

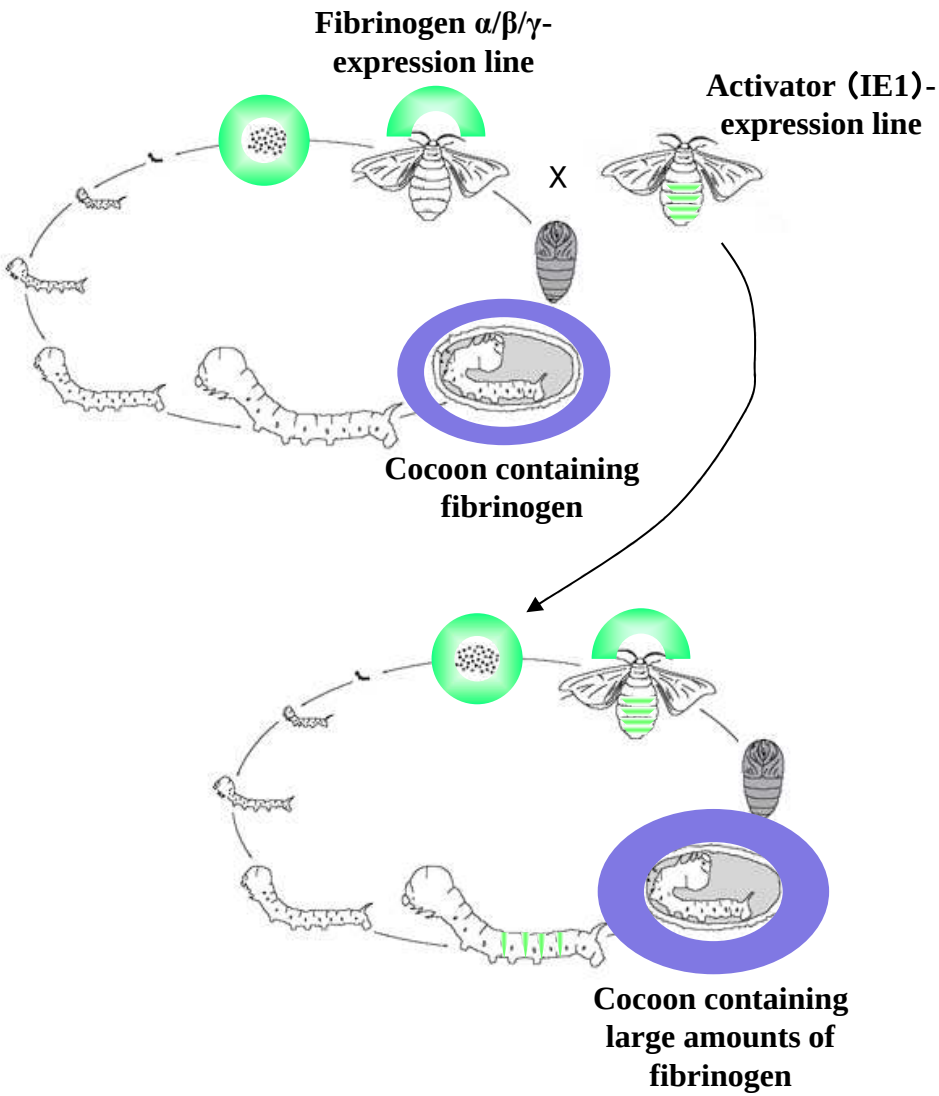


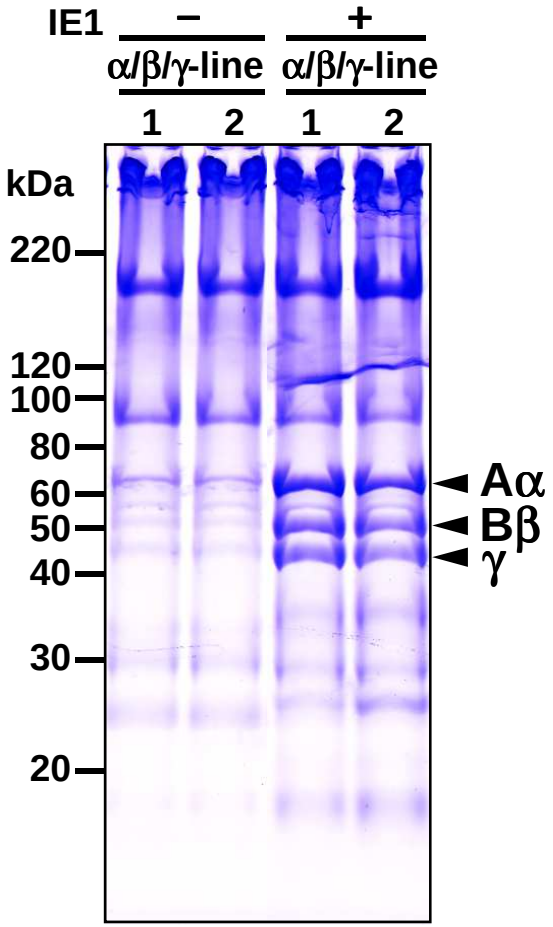

**Blood clotting cascade**




**Molecular structure of fibrinogen**

# Development of TG silkworm that produces human fibrinogen




**Amount expressed:**
**Approximately 2mg/cocoon**

# Hemostatic activity of recombinant fibrinogen

**Cocoons (α/β/γ＋IE1)**

↓

**Extraction of fibrinogen with 2 M urea, 0.1% TritonX-100, and 50 mM Tris-HCl (pH7.5)**

↓

**Concentration by ultrafiltration**

↓

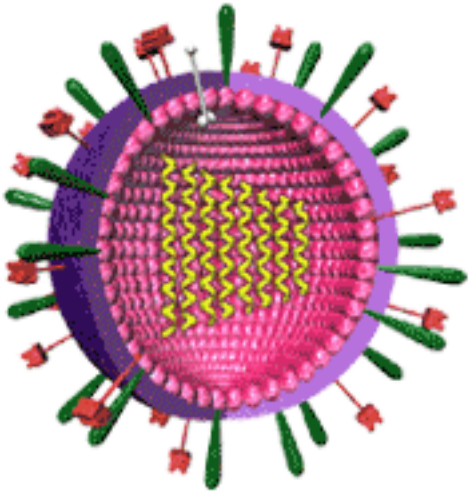
**Addition of 200 mM NaCl and 500 nM $CaCl_2$**

↓

**Incubation with 10 U/ml thrombin at 37℃ for 1 hr**

**Silkworm-produced recombinant fibrinogen showed apparent hemostatic activity.**

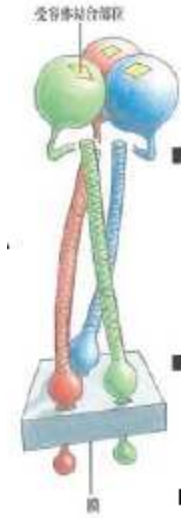
# Design of HA genes for expression in TG silkworm system



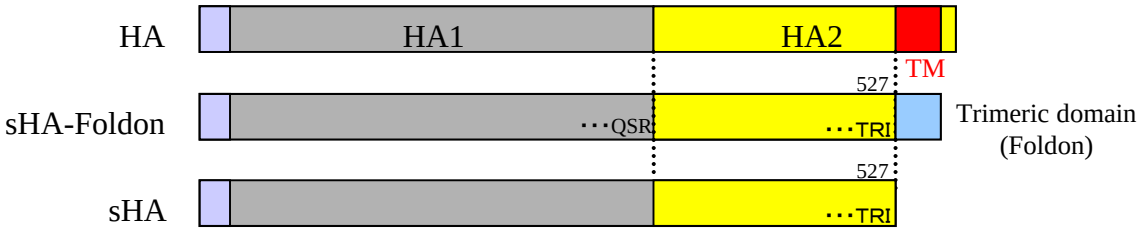
**HA (Hemagglutinin)**

**NA (Neuraminidase)**

**Structure of Influenza virus**

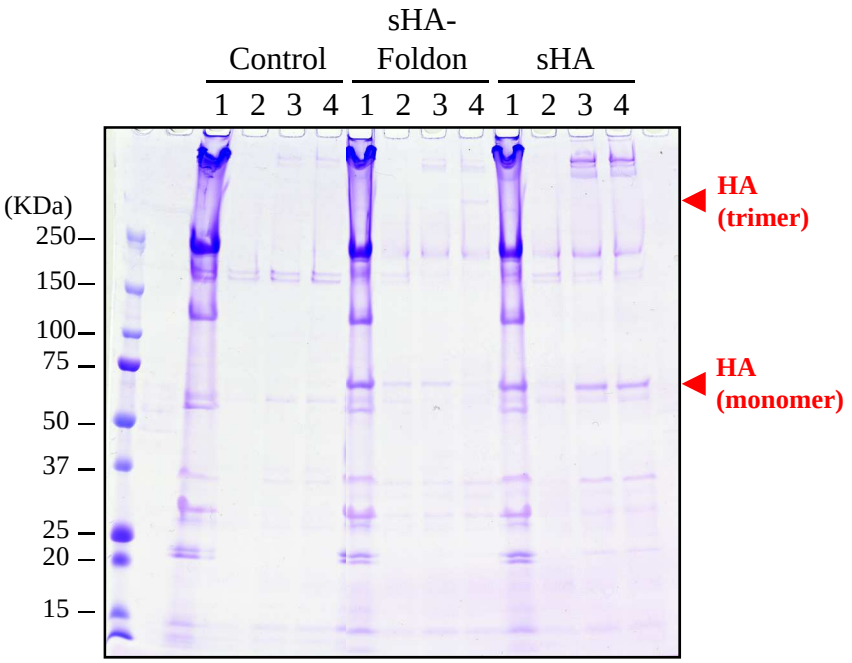


**Structure of Hemagglutinin**

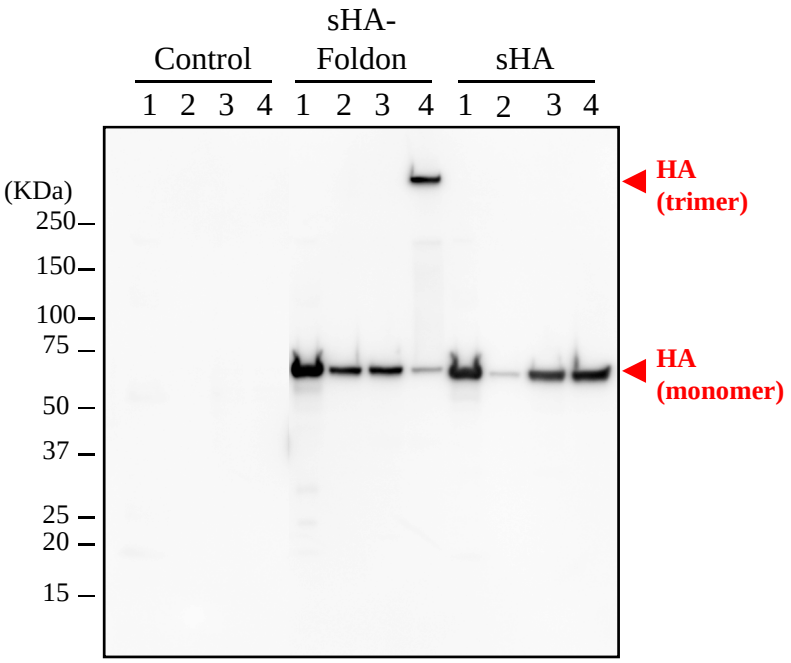



**Structures of HA genes for expression in TG silkworm system**
**(H1N1（A/Okinawa/248/2009）)**

# SDS-PAGE and Western blot analyses of transgenic silkworm cocoons




CBB

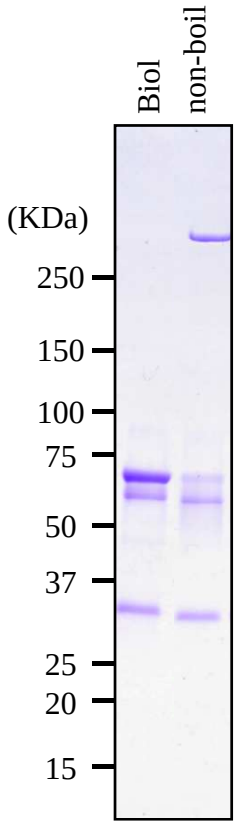



Anti-H1N1 antibody

1: Total extraction (8M urea, 50mM Tris(8.0), 2% 2-ME)
2: PBS (final 0.5MNaCl)
3: 0.1% Triton X-100 in PBS (final 0.5M NaCl)
4: 0.1% Triton X-100 in PBS (final 0.5M NaCl) Non-boiled

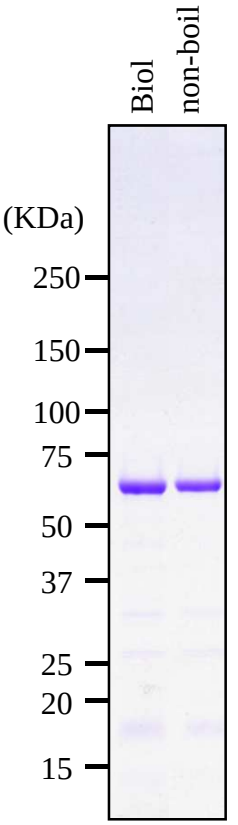
# Purification of recombinant HAs

**Cocoons**
↓
**Extraction with 0.1% Triton X-100 in PBS (final 0.5M NaCl)**
↓
**Ammonium sulfate precipitation**
↓
**Cation exchange chromatography**




**sHA-foldon**

**Cocoons**
↓
**Extraction with 0.1% Triton X-100 in PBS (final 0.5M NaCl)**
↓
**Ammonium sulfate precipitation**
↓
**Anion exchange chromatography**




**sHA**

# Antibody responses of recombinant HAs

**Immunization of ddY mice with about 30 μg of purified sHA-foldon or sHA**

↓

**Boost immunization 14 days and 21 days after first immunization**

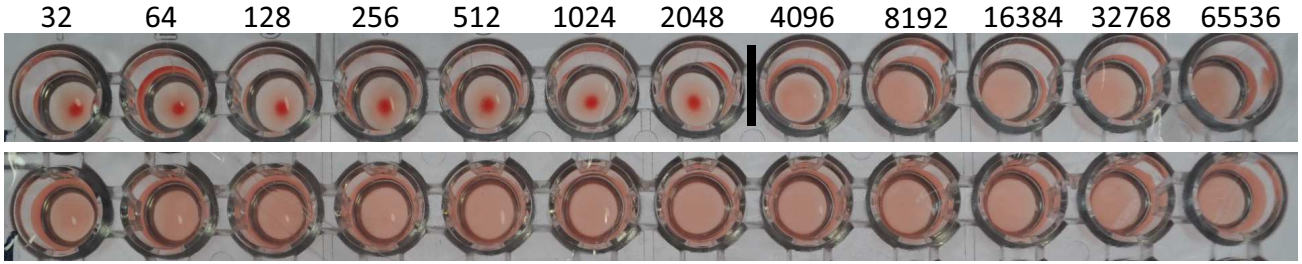
↓

**Collection of serum 28 days after first immunization**

↓

**Measurement of antibody titer by Hemaggultination Inhibition Assay (HAI)**

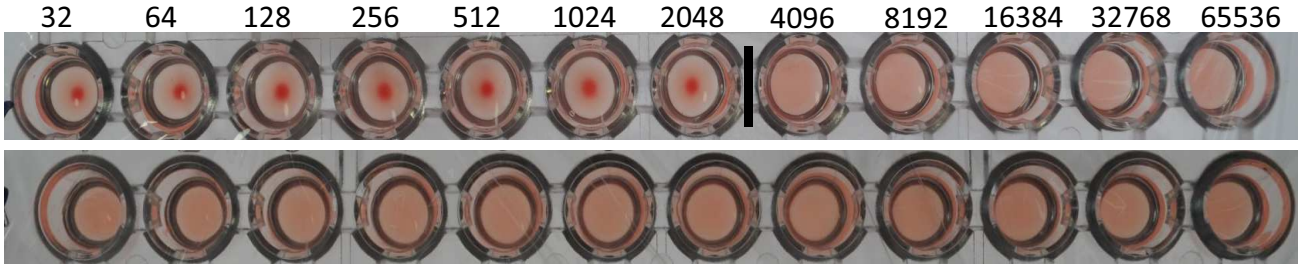
## sHA-foldon




**H1N1 (A/okinawa/248/2009)**
**HI titer: 2048**

**H5N1 (A/duck/singapore-Q/F19/3/97)**
**HI titer: Negative**

## sHA




**H1N1 (A/okinawa/248/2009)**
**HI titer: 2048**

**H5N31(A/duck/singapore-Q/F19/3/97)**
**HI titer: Negative**

**High antibody responses were observed by immunization with both sHA-foldon and sHA.**