FINAL REPORT

C O N F I D E N T I A L

Transient Expression and Purification of Recombinant GP

Project Order # 340397-1

Date: 01/27/2014

This report is provided by:

GenScript USA Inc
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Piscataway, NJ 08854
United States of America

This study was conducted according to the procedures described in this report. All data presented are authentic, accurate and correct to the best of our knowledge. Information and materials provided by this investigation are for ***research use*** only.

SIGNATURE

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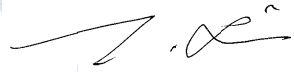

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1. Project Summary

This report describes the transient expression of GP protein in suspension 293-6E cells using serum free medium, followed by one-step purification from both cell culture supernatants and total membrane proteins extracted from cell lysate.

2. Experimental Methods

2.1 Cell Culture and Transient Transfection

293-6E cells were grown in serum-free FreeStyle™ 293 Expression Medium (Life Technologies, Carlsbad, CA, USA). The cells were maintained in Erlenmeyer Flasks (Corning Inc., Acton, MA) at 37°C with 5% CO_2 on an orbital shaker (VWR Scientific, Chester, PA). One day before transfection, the cells were seeded at an appropriate density in Corning Erlenmeyer Flasks. On the day of transfection, DNA and PEI (Polysciences, Eppelheim, Germany) were mixed at an optimal ratio and then added into the flask with cells ready for transfection. Cell pellets and cell culture supernatant were collected at day 4 post-transfection for further purification.

2.2 Purification and Analysis

Cell culture supernatant and total membrane proteins extracted from cell lysate were loaded onto Ni Sepharose 6 FF 0.3 mL resin (GE Healthcare, Cat.No.17-5318-02), respectively. After washing and elution, the purified protein was analyzed by SDS-PAGE and Western blot by using standard protocols for molecular weight and purity.

3. Experimental Results

3.1 GP purified from cell culture supernatant

The plasmid DNA encoding GP was transiently transfected into 100 mL suspension 293-6E cells. Cell supernatants were collected at day 4 post-transfection and the target protein was purified by loading onto Ni Sepharose 6 FF 0.3 mL resin. The samples from purification step were analyzed by SDS-PAGE and Western blot analysis as shown in Figure 1. The

primary antibody for Western blot was Mouse-anti-his mAb (GenScript, Cat.No.A00186).

A. SDS-PAGE **B. Western blot**

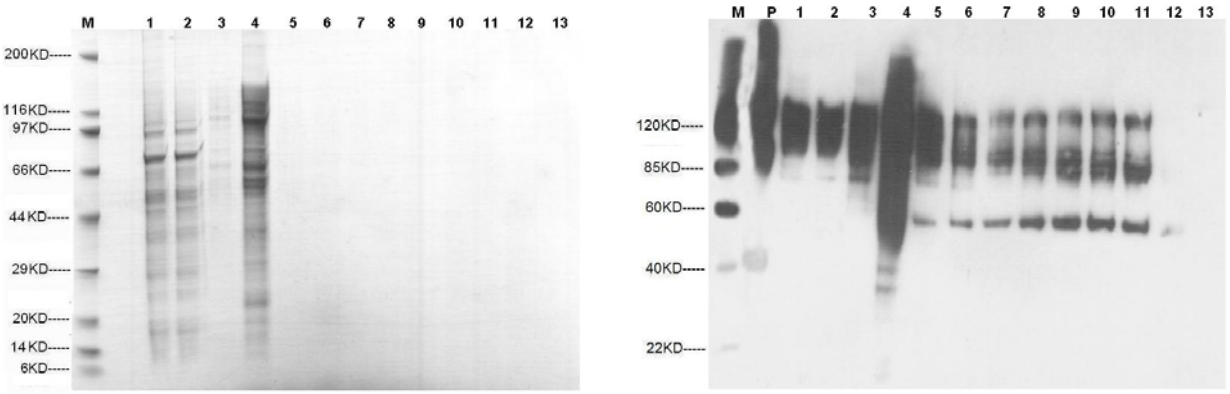

Figure 1. SDS-PAGE and Western blot analysis of day 4 cell culture supernatant after one-step purification

Lane M: Marker
Lane 1: Cell culture supernatant after centrifugation
Lane 2: Flow-through
Lane 3~4: 50mM imidazole eluted fractions
Lane 5~7: 100mM imidazole eluted fractions
Lane 8~11: 250mM imidazole eluted fractions
Lane 12~13: 1M imidazole eluted fractions
Lane P: Multiple-tag (GenScript, Cat.No.M0101) as positive control

We have tried to purify the target protein GP from cell culture supernatant. However, very little protein could be detected in eluted fractions. It indicated that the protein was positively expressed in suspension 293-6E cells. However, the target protein was not bound well onto the resin.

3.2 GP purified from cell lysate

Cell lysate collected from day 1 to day 4 post-transfection were analyzed by SDS-PAGE and Western blot as shown in Figure 2. The cells were harvested at day 4 post-transfection, lysed to collect target protein. The extracted membrane proteins were loaded onto Ni Sepharose 6 FF 0.3 mL resin to capture target protein, and analyzed by SDS-PAGE and Western blot as shown in Figure 3 ~ Figure 4. The primary antibody for Western blot was Mouse-anti-his mAb (GenScript, Cat.No.A00186).

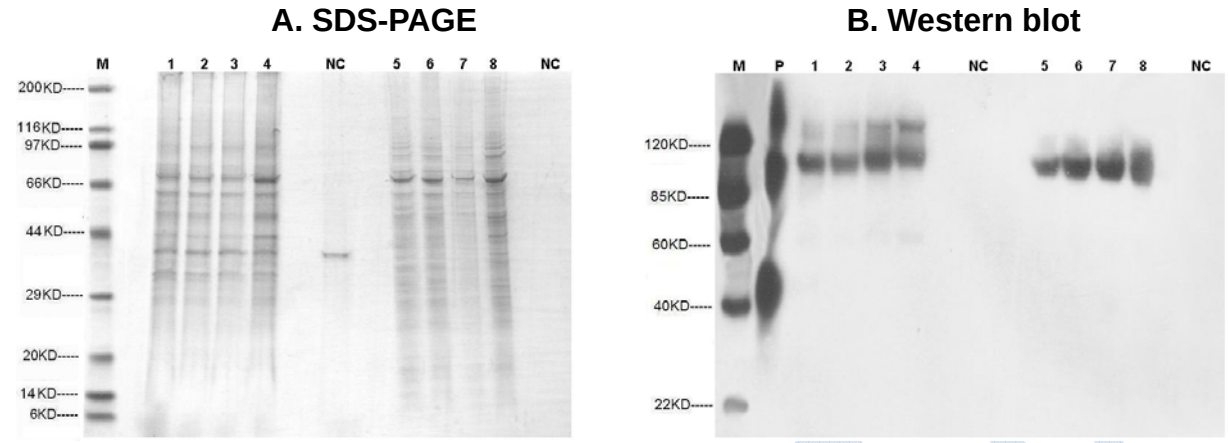

Figure 2. SDS-PAGE and Western blot analysis of cell lysate from day 1 to day 4 post-transfection

Lane M: Marker
Lane 1~4: Cell lysate from day 1 to day4 post-transfection with RIPA buffer
Lane 5~8: Cell lysate from day 1 to day4 post-transfection with hypotonic buffer
Lane NC: Negative control
Lane P: Multiple-tag (GenScript, Cat.No.M0101) as positive control

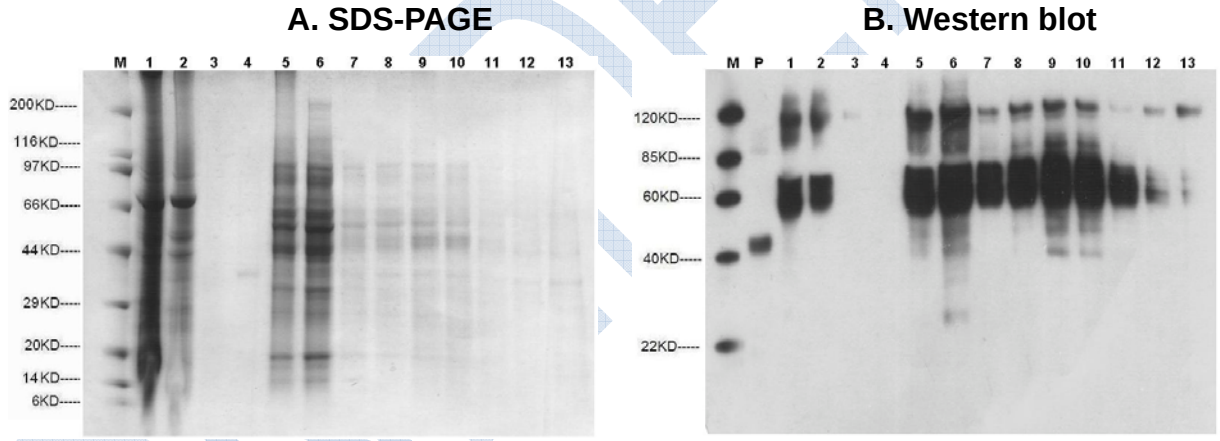

Figure 3. SDS-PAGE and Western blot analysis of GP from day 4 cell lysate after one-step purification

Lane M: Marker
Lane 1: Cell lysate supernatant
Lane 2: Flow-through
Lane 3~4: 50mM imidazole eluted fractions
Lane 5~8: 100mM imidazole eluted fractions
Lane 9~12: 250mM imidazole eluted fractions
Lane 13: 1M imidazole eluted fraction
Lane P: Multiple-tag (GenScript, Cat.No.M0101) as positive control

The fractions containing purified GP were collected and desalted to PBS, with 0.1% DDM,

pH 7.2. The final products were analyzed by SDS-PAGE and Western blot analysis as shown in Figure 4. The primary antibody for Western blot was Mouse-anti-his mAb (GenScript, Cat.No.A00186).

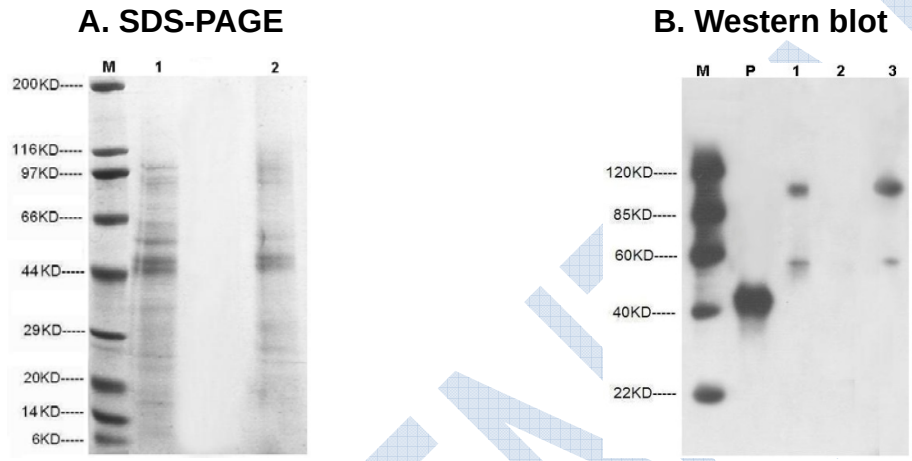

Figure 4. SDS-PAGE and Western blot analysis of purified GP from cell lysate after desalting

Lane M: Protein Marker
Lane 1: Reducing conditions
Lane 2: Non-reducing conditions
Lane P: Multiple-tag (GenScript, Cat.No.M0101) as positive control

4. Conclusion and Discussion

We have successfully expressed and purified GP in 293-6E cells from cell lysate. The purified protein was detected with estimated molecular weight of ~100 kDa (Cal.M.W. ~71.67kDa) on Western blot. 0.02 mg GP (Concentration: 0.2 mg/mL, Purity: ~50%) were delivered.

5. Packing List of Order 340397-1

Protein from cell lysate

(Shipping condition: -196℃)

- Name: GP
- Concentration: 0.2 mg/mL
- Volume per vial: 0.1 mL
- Purity: ~50%

- Buffer: PBS, with 0.1% DDM, pH 7.2
- Lot No.: 340397S1/B0491401
- Total protein: 0.02 mg
- Number of vials: 1
- Store at -80℃

6. Product Information

Order Number:	340397-1
Protein Name:	GP
Fusion Tag(s):	N-His6
Lot Number:	340397S1/B0491401

Codon Optimization:	Yes
Expression System & Vector:	293-6E cells, Mammalian Expression Vector pTT5
Expression Optimization:	Yes
Expression Scale:	100 mL
Tag Removal:	No
Purification:	Protein was obtained from cell lysate, followed by one step purification by Ni Sepharose 6 FF resin

Package:	0.02 mg, 0.1 mL/vial, 1 vial
Concentration:	0.2 mg/mL as determined by Bradford assay
Purity:	About 50% as estimated by densitometric analysis of the Coomassie Blue-stained SDS-PAGE gel
Storage and Handling:	Store at -80°C. Aliquots should be stored at the same temperature after first use to avoid multiple freeze-thaws
Storage Buffer:	PBS with 0.1% DDM, pH 7.2

Appendix

Cloning Strategy:

EcoRI -- Kozak sequence -- ATG -- Artificial Signal Peptide -- 6xHis -- Target Protein Envelope glycoprotein (33-671 aa optimized for HEK293) -- stop codon -- HindIII

DNA Sequence:

2034 bp

```
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Protein Sequence:

669 aa

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