Recombinant Sip Young 6 His Protein Production from the Sea Star Igkappa Gene, after Cloning

Michel Leclerc

ABSTRACT

In 2014 we have isolated and cloned the sea star Igkappa gene which induced an anti-HRP primitive invertebrate antibody. We attempt, in the present work, for the first time in Invertebrates, to produce a SIP Young 6 His protein through HeK 293 EBNA cells.

Keywords: Invertebrates; HEK 293 cells; sea star Igkappa gene; Asterias rubens.

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Michel Leclerc *
Immunology of Invertebrates, France.
(e-mail: mleclerc45@gmail.com)

**Corresponding Author*

I. INTRODUCTION

The sea star IGHK gene was discovered in 2014 [1]. It is an IPA (Invertebrate Primitive Antibody) which produce an anti-HRP protein. We recall it was obtained from sea star *Asterias rubens* immunized to HRP (Horse-radish peroxidase). In 2014 [2] it was inserted in an *Escherichia coli* plasmid and showed a specific binding to the antigen HRP [2].

In the present time we attempt to use another “carrier” to express this protein: The Hek 293 EBNA cells.

II. MATERIALS AND METHODS

A. Subcloning in Expression Vector

The genes coding for the target proteins were chemically synthesized with optimization for expression in HEK293 cells. The sequence is illustrated below [1].

B. Sequences Information

> SIP_Young_6His cDNA - 384 bp

ATGGGCGGAATGAGAGGCAACATGGCCAGCCTG
TGGATGTTCTTTTCGTGGTGGGCATCACACTGCAG
AGGAGC

CTGGCCATCTACACATTGAGAGAGCAGCCTTCCG
ACACCTCCGCCCTGCAGGGCAGCACAGTGGTGCTG
CACTGT

AGCGTGGAGCAGTACATCAACACCACCGCCATC
GTGTGGTGGAGCAGGGATTCCGTGATCTCCACAA
CAACGAC

CTGAACCTGAGCAGCCTGAACACAGATCAGCTGCA
GAGCTACAGCATCAGCGGCGACGCCTCCAGGGGCG
AGTTC

AATCTGAATATCGTGAATTTACAGCCACCGATGC

CGCCAGCTACAGATGTCAGATGCTGGAGCACCACC
ACCAC

CATCACTGA

Expected protein sequence

> SIP_Young_6His – 127 AAs – 14.30 kDa

MGGMRGNMASLWMFFVVGITLQRLAIYTFREQ
PSDTSALQGSTVVLHCSVEQYINTTAIVWWSRDSVIS
HNND

LNLSSLNTDQLQSYSISGDASRGFNLNIVNFTATD
AASYRCQMLEHHHHHH Features:

SIP: [1:27]

His tag [122:127].

III. RESULTS

Small-scale Expression and Purification Tests

A. Short Protocol Description

The pTXs2 construction obtained as described in §2 were transfected in HEK293 cells (80 ml culture) by using NeoBiotech's proprietary Xten transient transfection protocol. Culture medium and cell samples were collected until viability drops under 50% post-transfection. 1.5 ml Cell culture were collected, followed by semi-purification with Ni resin and SDS-PAGE with WB analysis. The results are illustrated below.

Reduced SDS-PAGE analysis after sonication of cells, then western-blots were obtained in Fig. 1.

It shows that the protein has a molecular weight of about 14 Kd after denaturation in PBS pH 7.5 with urea 8 M.

Fig. 1. Expression test result.

A – Reduced SDS-PAGE analysis. Coomassie blue staining.

B – Western blot with anti-his antibody (ECL revelation).

o. Negative control culture. Medium: Analysis of the culture medium.

NPE: Native protein extracts obtained by cell lysis (sonication) in native buffer (PBS pH 7.5).

DPE: Denatured protein extracts prepared by solubilization of the pellet obtained after the native lysis in a denaturing buffer (PBS pH 7.5 with urea 8 M).

B. Short Purification Protocol Description

Culture medium was purified by affinity against His-tag (IMAC) by a standard method:

- Equilibration with PBS, pH 7.5
- Wash 1/2/3 with PBS, pH 7.5, 0 mM, 30 mM and 50 mM imidazole buffer
- Elution with PBS, pH 7.5, 200 mM and 400 mM imidazole buffer
- Analysis by SDS-PAGE of fractions of interest
- Final sample QC: qualitative by SDS-PAGE, quantitative by Bradford method

E2-E6 were pooled, buffer exchanged vs PBS, pH7.5 by dialysis method. The purification test results, and QC are illustrated in Fig. 2 and Fig. 3.

Fig. 2. Target protein purification profile. Coomassie blue staining.

A. Reduced PAGE analysis.

B. Western blot with anti-his antibody (ECL revelation).

MW – Molecular weight marker. IN – Input. FT – Flow through.

W1-W3 – Washes. E – Eluted fractions.

Fig. 2. The final QC gel. Coomassie blue staining.

A. Reduced PAGE analysis.

B. Western blot with anti-his antibody (ECL revelation). MW. Molecular weight marker.

These figures show clearly that the eluates E2, E3, E4 contain mainly the SIP Young 6 His protein which is revealed by the anti-His antibody (ECL revelation).

IV. CONCLUSION

We retain the following characteristics of the obtained SIP Young 6 His protein

The final buffer (PBS, pH 7.5) has a final quantity of 0.39 mg for a concentration of:0.13 mg/ml. We note also that the yield is: 4.88 mg per liter of culture: that is low. But it is the first time, at our knowledge an invertebrate IGHKappa gene is inserted in HeK 293 cells.

The SIP Young 6 His protein possess a great future. It constitutes the first invertebrate protein with immune properties at least in invertebrates. Currently we study its potentialities in vertebrate immune functions.

REFERENCES

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- [2] Leclerc, M. and Otten, P. (2014) S. A. J Biotechnol. 1:104-105.