

The Sea Star Igkappa Cloning by the use of e. Coli and Cho Protocol

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Received date: November 13, 2023; **Accepted date:** December 01, 2023; **Published date:** January 29, 2024

Citation: Michel Leclerc, (2024), The Sea Star Igkappa Cloning by the use of e. Coli and Cho Protocol. *J. Pharmaceutics and Pharmacology Research*, 7(2); DOI:10.31579/2688-7517/157

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Abstract

This previous work has performed with UMR-INRA ISP1282 (Nicolas Aubrey). It allows, by the use of CHO (Chinese Hamster Ovarian) protocol cloning to produce in a relative great quantity the « Young Protein » or anti-HRP (Horse-Radish Peroxydase) from the sea star IGKappa gene

Key words: protocol; protein; production

Introduction

10 years ago, we tried to clone, for the first time, the sea star IG Kappa gene by the use and the help of E. coli as amplificador [1]. It allowed, in a second time, to verify that the Young Protein, or anti-HRP Protein recognizes the HRP antigen [2].

But, this verification of the affinity between the IPA (Invertebrate Primitive Antibody) and the antigen, seemed unclear at that time, for many of us. Thus, we decided to operate new cloning [3] with new parameters and new affinity tests.

The last of them, used CHO protocol, as described in various experiments [4].

Results:

First, the percentage of Young Protein production, was greatly ameliorated with the CHO protocol. A best rate occurs when compared to the E. coli one sensu stricto. A western-blot determined exactly the M.W of the Young Protein, as shown in Figure 1: It is 12,49271 Kda.

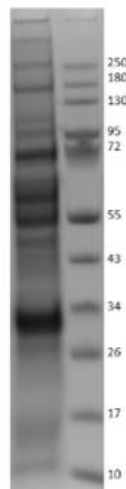


Figure 1 : Purification of Young Protein (Production en ExpiCHO)

Discussion:

We think now to perform an Elisa test to verify the affinity between this young protein and the HRP antigen.

We envisage also (My colleagues and me) in a next future, to immunize other sea stars with anti-tumoral antigens to product specific nanobodies (Ref.4) from sea stars, against cancer activity (in a general way):

sequencing and cloning, after'll be applicated to obtain a specific recombinant specific protein.

Références:

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2. Leclerc, M. (2023) J Stem Cell Res Ther 8(1) :25
3. Ryu, J. et al (2022) BMC Biotechnol 22 :8
4. Leclerc, M. (2022) Mathews J Immunol Allergy 6(1) :14



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DOI: [10.31579/2688-7517/157](https://doi.org/10.31579/2688-7517/157)

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