Scientific Research Publishing

Journal of Biosciences and Medicines, 2016, 4, 31-36
http://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

# The Determination and Evaluation of the Biological Activities for the Commercialization of Recombinant Follicle-Stimulating Hormone *in Vitro*

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**How to cite this paper:** Hu, J.W., Han, J.Z., Zhang, X., Chen, F., Liu, L.L. and Zeng, B. (2016) The Determination and Evaluation of the Biological Activities for the Commercialization of Recombinant Follicle-Stimulating Hormone *in Vitro*. *Journal of Biosciences and Medicines*, **4**, 31-36.
http://dx.doi.org/10.4236/jbm.2016.412005

**Received:** October 12, 2016
**Accepted:** November 24, 2016
**Published:** December 1, 2016

## Abstract

Follicle-stimulating hormone (FSH) plays a central role in mammals reproduction, with the actions of FSH mediated by follicle-stimulating hormone receptors (FSHRs) on the surface of target cells. The purposes of this study were to determine and evaluate the biological activities for the commercialization of recombinant follicle-stimulating hormone (rFSH) *in vitro* through the cellular internalization using cloned 293T-FSHR cell lines as target. Using imaging approaches we have found here that a little fluorescent signal from the surface of the cell transferred to the cytoplasm and accumulated around the nucleus by endocytosis. Compared with the control groups, the commercialization of rFSH have not the significant differences of internalization, but the rFSH have promoted the internalization of the fluorescent, suggested that this detection system might as a protocol for the bioactivity of recombinant therapeutic proteins *in vitro*.

## Keywords

Recombinant Follicle-Stimulating Hormone (rFSH), Follicle-Stimulating Hormone Receptor(FSHR ), Cellular Internalization

## 1. Introduction

G protein-coupled receptors (GPCRs) are a superfamily of cell-surface receptors which modulate a variety of cell functions through coupling to heterotrimeric G-proteins and regulating downstream effectors such as adenylyl cyclases, phospholipases, protein kinases and ion channels [1]. FSHR belongs to the subfamily of GPCRs, which have great

potential as therapeutic targets for abroad spectrum of diseases [2] [3] [4]. Follicle-stimulating hormone (FSH) was an important glycoprotein hormone to regulate reproduction in mammals, it acts through binding to follicle-stimulating hormone receptor (FSHR) on target cell surfaces [5] [6]. The biological activities and half-life of FSH are associated with glycosylation, sialylation and sulfation [7] [8].

As the biotechnology has rapidly expanded in recent years, the majority of recombiant proteins have been produced by the genetic engineering technique using different host cell systems for the expression of heterologous genes such as *Escherichia coli*, Yeast, Insect/baculovirus, Mammalian cells, as well as Eukaryotic individuals, both of which have inherent advantages and drawbacks [9] [10]. Besides, a variety of proteins analogues have been obtained via chemical synthesis [11] [12] [13].

However, the bioactivity and physicochemical characteristics of the recombiant protein are not clearer *in vitro* or *in vivo*. At present, the mainly methods to detect the *in vitro* bioactivity for the recombinant follicle-stimulating hormone by the rat granulosa cell or the Sertoli cell aromatization bioassays [14] [15] and follicle-stimulating hormone radioreceptor activity methods [16]. In this study, we have adopted a methods of the pSNAPf-ADR$\beta$2-FSHR [17] and cellular internalization [18] to determination and evaluation for the biological activities of recombinant follicle-stimulating hormone *in vitro*.

## 2. Materials and Methods

### 2.1. Reagents and Standards

The cloned human kidney 293T-FSHR cell strains (our laboratory), 96-well cell culture plates (NEST), SNAP-Surface™ 549 (NEB), DMEM (Hyclone), Fetal bovine serum (WISTEN), Bovine serum albumin(BSA, Sigma-Aldrich; USA), Puregon (Organon, Netherlands), Carbon dioxide incubator(Thermo), Fluorescence microscope (Bio-Rad).

### 2.2. The Cellular Internalization for rFSH *in Vitro* Activity Assay

A HEK-293T cell lines that had been stably transfected with the pSNAPf-ADR$\beta$2-FSHR was used to detect fluorescent signal in response to the commercialization of recombinant follicle-stimulating hormone (rFSH) stimulation. Fluorescent signal were determined using imaging approaches, the total process of the cellular internalization as follows.

Cells were cultured in 96-well plates with 200 ul of DMEM supplemented with 10% fetal bovine serum (FBS). After 48-72 hours, replace the medium with 50 ul/well the labeling medium of 5 μM SNAP-Surface 549 (NEB) containing 0.5% bovine serum albumin (BSA) and incubate at 37˚C, 5% $CO_2$ for 15 minutes. Wash the cells ten times with DMEM (Hyclone) containing 10% fetal bovine serum (FBS, WISTEN) and PBS (0.01 M, PH7.2), respectively. Adding 50 ul/well Puregon dilution (30 mIU/ml) and set the controls, respectively. Image the cells using the Fluorescence microscope (Bio-Rad) and continuously recording 0 min, 30 min, 60 min, 90 min, 120 min, 150 min.

## 3. Results

The cellular internalization for rFSH *in vitro* activity assay, we have found here that a little fluorescent signal from the surface of the cell transferred to the cytoplasm and accumulated around the nucleus. Compared with the control groups, the commercialization of rFSH have not the significant differences of internalization. The control groups (**Figure 1**), after 90 min appeared a large number of fluorescence accumulated around the nucleus. However, the experimental groups (**Figure 2**), after 60 min have appeared the fluorescence accumulated around the nucleus. Suggested that the recombinant follicle-stimulating hormone promoted the fluorescent from the surface of the cells into the cytoplasm and accumulated around the nucleus. Meanwhile, the results indicated that this detection system was successed in determination and evaluation of the biological activities for rFSH *in vitro*.

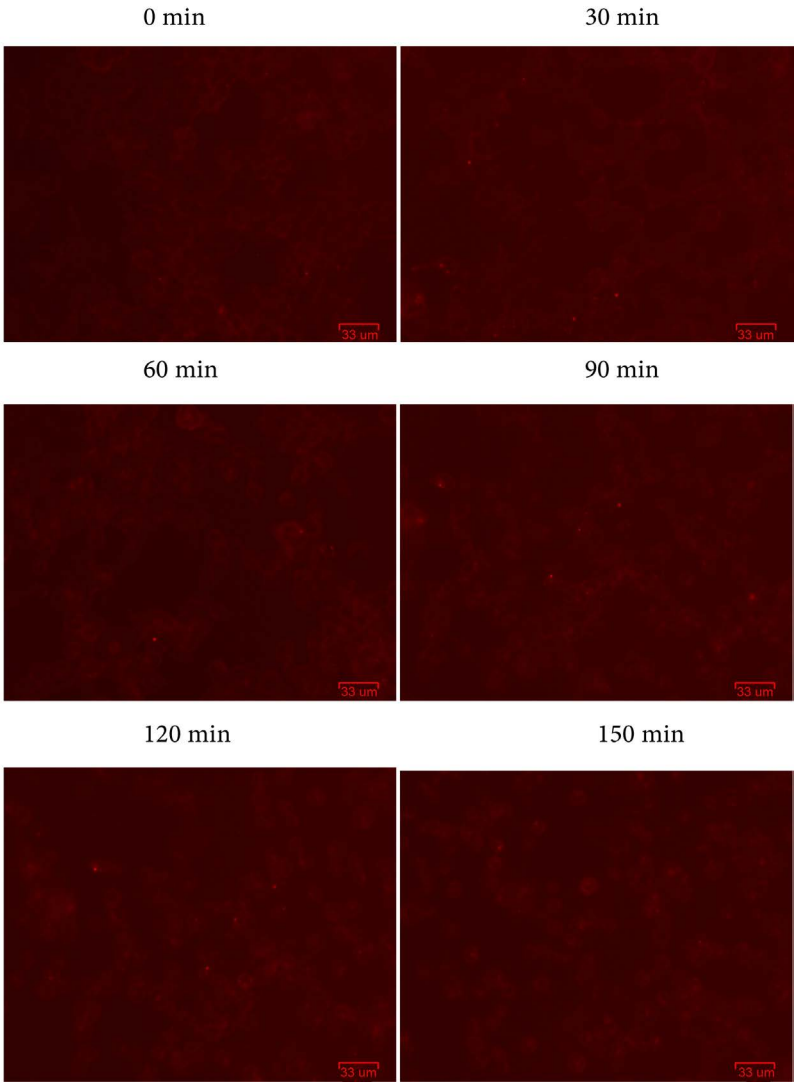


**Figure 1.** The control goroups: 50 ul/well PBS, images acquisition from 0 min to 150 min.

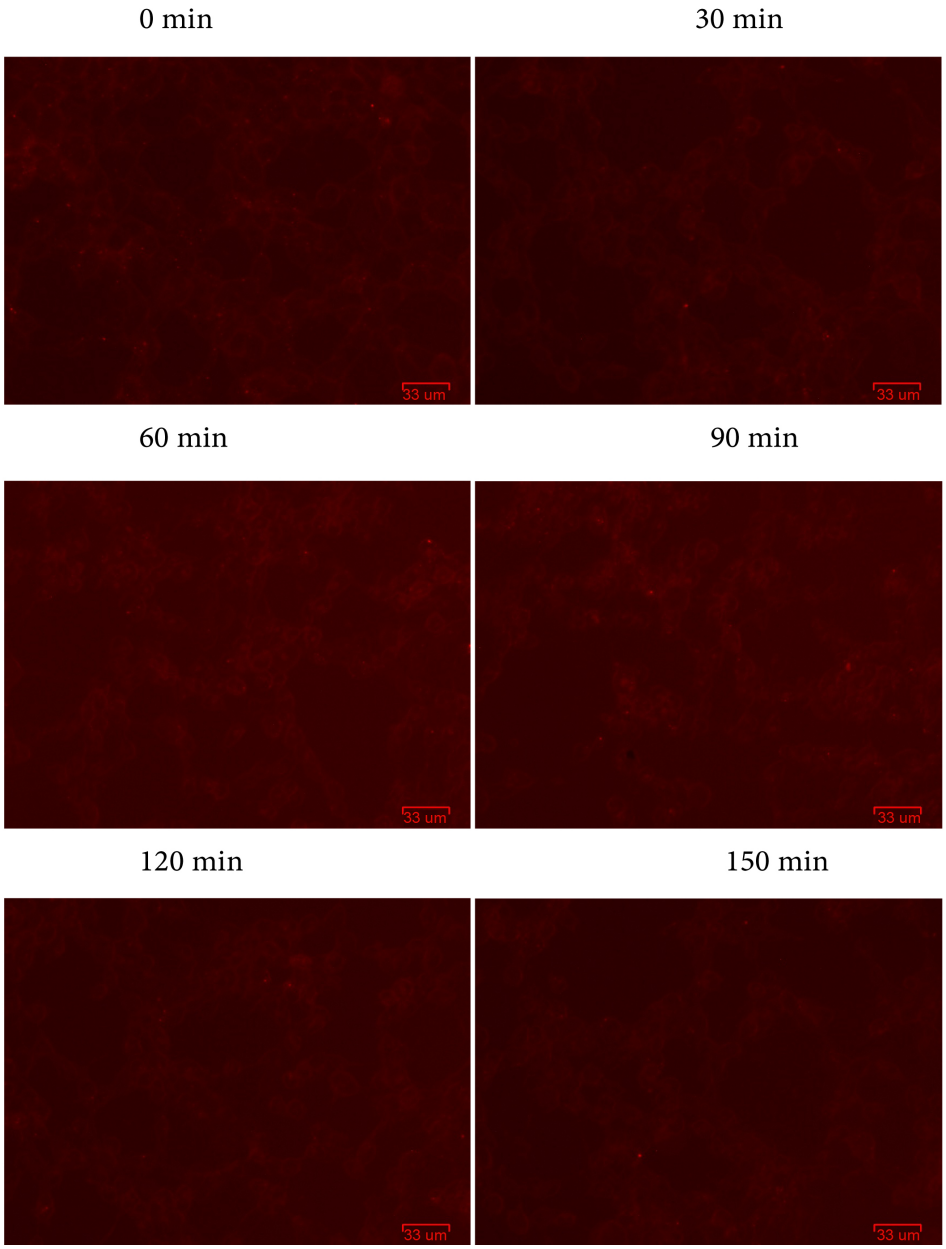


**Figure 2.** The experimental groups: 50 ul/well pruegon dilution (30 mIU/ml), images acquisition from 0 min to 150 min.

## 4. Discussion

The detection system based on the FSH receptor activation by FSH, FSHR induces intracellular cAMP synthesis, in turn, triggers downstream signal transduction [19] [20]. To reduce background interference, used 0.5% bovine serum albumin (BSA) in the labeling medium and removed the SNAP-Surface 549 dye with DMEM containing 10% fetal bovine serum (FBS). Compared with the control groups, the commercialization of the rFSH have promoted the internalization of the fluorescence, suggested that this detection system might as a protocol for the bioactivity of recombinant therapeutic

proteins *in vitro*.

However, there are also many drawbacks such as low sensitivity, inconvenience in tracing images, and unsatisfied in recognising result. In the next study, we will further improve the detection system of bioactivity for rFSH and combined with cAMP-Screen cAMP Immunoassay System [21] [22].

## Acknowledgements

This work was supported by these projects in China (31171731, 31460447, 20142-BDH80003, 2013-CXTD002, "555 talent project" of Jiangxi Province, Jiangxi Province Key Laboratory of Bioprocess Engineering and Co-Innovation Center for *in vitro* Diagnostic Reagents and Devices of Jiangxi Province).

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