The experiment procedure for a study assistant: Pitfalls of experimental kits

Miki Fujimoto[#1], Kayo Nagahama[#1], Yukiko Kuroda[#1]

#1 . Department of Clinical Resarch, Tokushima National Hospital, National Hospital Organization, 1354 Shikiji, Kamojima, Yoshinogawa, Tokushima 776-8585 Japan

Abstract

In Tokushima National Hospital, we primarily conduct clinical studies about nerves, myopathy and fundamental research in the. We study the molecular mechanism of the neurodegenerative disease that we did including Parkinson's disease as fundamental research in the clinical Research Department to which I belong. I work on fundamental research every day. In recent years the experiment procedures have progressed drastically. A simple and easy kit is sold by the companies. However, we often find that the results that we expected even if we believe a kit to be of good quality and have tested it did not appear. We herein report some cases.

Key words: experiment procedure, study assistant, pitfall, experimental kits

Introduction

In Tokushima National Hospital we primarily conduct clinical studies about nerves, myopathy and fundamental research. The researchers conduct fundamental research in the clinical Research Department to which I belong. Specifically, the researchers study the molecular mechanism of neurodegenerative disease that we did including Parkinson's disease. I work on fundamental research every day. In recent years the experiment procedures have progressed drastically. A simple and easy kit is sold about the state-of-the-art by the companies. However, it is not unusual to fail even if we use a kit. We report some cases this time.

Materials and Methods

Kit 1: In-FusionR HD Cloning Kit® (TAKARA BIO INC., Tokyo, Japan)
In-Fusion HD Cloning Kits are designed for fast, directional cloning of one or more fragments of DNA into any vector. We tested this kit according to the manufacturer's instructions. We added 15 bases which were homogenous to 5' end of the primer for the purpose of gene amplification to the end sequence of the vector. We amplified a target gene with the primer. The sequence of the primer which we used was as follows.

Fragment 1 FW Primer: TAC ATC AAG GCG ATG GCA GAA ATC GGT ACT G

Fragment 1 RV Primer: TGC AAT AAA CAA GTT CAC GTC GAA CCA GTG GTC

The amplified product was treated with Cloning Enhancer. After that, we purified the product from gel, we connected the DNA sample to a linearized vector using In-Fusion enzyme (In-Fusion response: at 50℃ for 15 minutes). The sample was transformed with an In-Fusion reaction. We finally screened a purpose clone.

Kit 2: Pyruvate Kinase Activity Colorimetric/Flurometric Assay Kit®
We measured pyruvate kinase activity using the Pyruvate Kinase Activity Colorimetric/FlurometricAssay Kit® (BioVison, California, USA). Using standard of the kit attachment, we drew a standard curve. We set up a response reagent of the kit attachment and adjusted the sample. We set up a response reagent of the kit attachment and added it to the sample. At room temperature, 30 minutes later, we measured optical density at 570nm.

Results

Kit 1: In-FusionHD Cloning Kit®
We picked up 233 colonies. We checked insert in colony PCR, but everything was negative (Figure 1). We give up this kit and it places an order outside and will build a vector after all.
Kit 2: Pyruvate Kinase Activity Colorimetric/Flurometric Assay Kit®
We were not able to draw even a standard curve. We used up 1 kit without being able to measure it.

Discussion

We tried kit 1 several times, but did not get along well with it. We sent this operation out after all. We did not succeed with kit 2 either. The problems considered are as follows, 1) Is the experiment procedure correct? 2) Is the sample adjustment appropriate? 3) Is the response Buffer appropriate? 4) Which is appropriate for measurement, an absorptivity plate leader or a spectrophotometer ? 5) Is it appropriate for the latent time? We wanted to try it in various ways, but the kit ran short. The kit was expensive but we purchased it again. As a result, we were able to measure the Kinase activity of the A protein (Figure 3). Sales messages as if results are obtained easily are used for a kit well. It is not appropriate that we depend only on the attached protocol of the kit. We reflected that we should use the kit on theoretical understanding of the background.

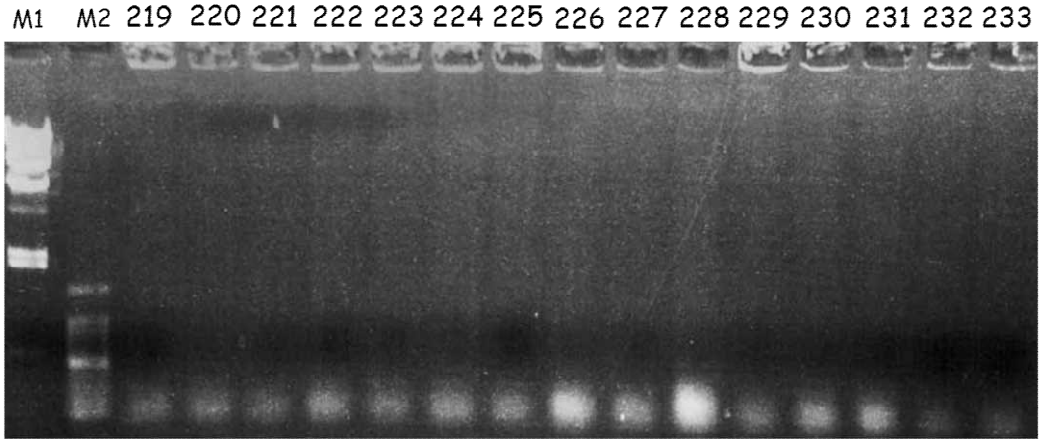

Figure 1. Results of cloning using the In-FusionHD Cloning Kit®. We picked up 233 colonies. We checked insert in colony PCR, but everything was negative.

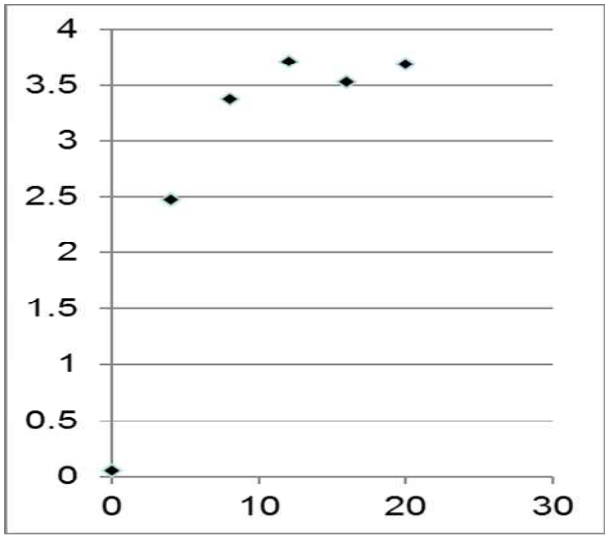

Figure 2. Results of colorimetric assay with using a standard sample. We were not able to draw even a standard curve.

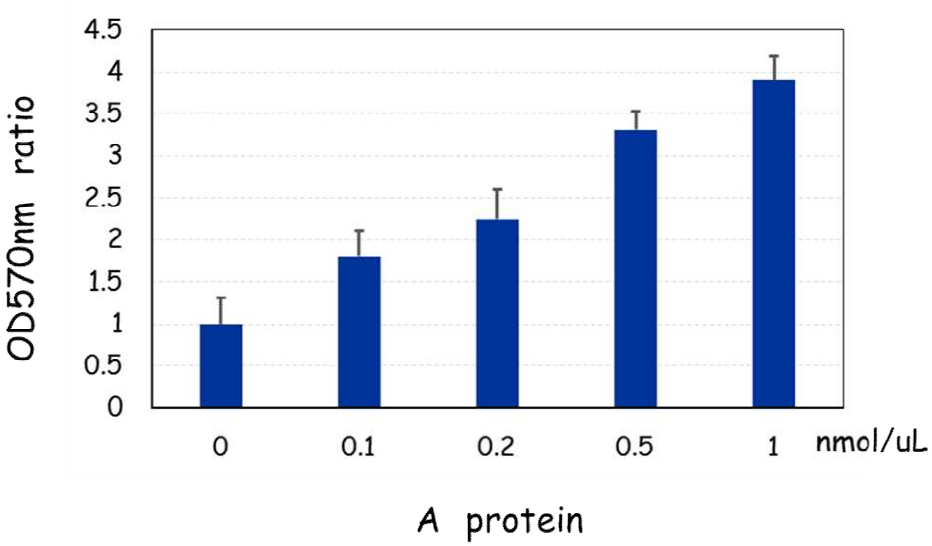

Figure 3. Results of colorimetric assay using a Pyruvate Kinase Activity Colorimetric /Flurometric Assay Kit® purchased again. We successfully assayed the activity of A protein.