A Comparative Assay for Hybridoma Supernatant Screening
Wei Hu1, Prudence Tsui1, Thomas Tang2, David Wong1, Walter Assuerer1 and Wilber Huang2
1Axela Biosensors, Inc., 480 University Avenue, Suite 910, Toronto, ON M5G 1V2 Canada, 2Abnova Corporation, 9th Fl., No. 108, Jhouzh District, Taipei City, 114 Taiwan

Abstract

In hybridoma screening, it is difficult to evaluate antibody affinities because of unknown IgG expression levels. Sometimes, endpoint results cannot distinguish between low affinity and high affinity antibodies. We have developed a simple assay to evaluate antibody expression levels and monitor real-time antibody-antigen binding. The assay employs a rabbit anti-mouse IgG-Fc surface to capture antibodies in hybridoma supernatants, which is followed by a buffer wash to eliminate antibodies that do not interact with the antigen. Antibodies are then ranked on expression levels and apparent binding affinity. We have developed a simple assay to evaluate antibody expression levels and monitor real-time antibody-antigen binding. The assay employs a rabbit anti-mouse IgG-Fc surface to capture antibodies in hybridoma supernatants, which is followed by a buffer wash to eliminate antibodies that do not interact with the antigen. Antibodies are then ranked on expression levels and apparent binding affinity.

Principles of Diffraction

Figure 1. Diffraction occurs because of the wave nature of light: when coherent light strikes a non-random pattern of obstacles, the resulting constructive and destructive interference produces a diffraction pattern. Capture molecules, such as antibodies, are immobilized in a specific pattern of lines on the surface of the prism-shaped dotLab™ Sensor. The sensor surface forms the base of a low volume fluidic controller. Binding of target molecules to the patterned molecules is monitored with a laser. Since illumination occurs through an optical prism, the laser beam does not pass through the solution in the flow channel.

Materials and Methods

dotLab Sensors: Polyethylene sensors immobilized with avidin were used.

Antibodies (Ab): Biotinylated rabbit-anti-mouse Fc (b-RAM-Fc), hybridoma supernatants and purified IgG, 1C5, 3D3, TE11 raised against ASCL1 and GST-conjugated rabbit or goat anti-GST (HRP-conjugated GST)

Antigen (Ag): Recombinant human ASCL1 fused to GST (GST-ASCL1)

Buffer System: HBS-Tween 20 (0.05% Tween).

Blocking Agents: Bovine serum albumin (BSA) (5 mg/mL), or DMEM with 5-10% casein

Procedures: Sequential addition of b-RAM-Fc (50 µg/mL), hybridoma supernatant (2/3 dilution with DMEM), GST-ASCL1 (10 µg/mL), HRP-conjugated GST (50 µg/mL), or purified GST-ASCL1 and HRP-conjugated GST (10 µg/mL, respectively).

Introduction

Optical biosensors can be used to monitor antibody-antigen interactions on surfaces in real-time without labeling the reactants, providing information about the reaction kinetics. Samples from crude preparations can be readily analyzed (Analytical Biochemistry (2004) 335, 381–387). Most often, antibody screening uses an antibody immobilized sensor surface.

The major concern is that the measured antibody activity is dependent on the unknown antigen concentration which makes it difficult to compare different antibody preparations. In addition, the activity is also affected by the bivalent antibody cross-link on the antigen surface (Talanta (2000) 53, 481–488).

Tagged fusion proteins as immunogens have a number of advantages including high stability, proper folding, high stability, and easy purification. However, antibodies raised against the fusion proteins sometimes are subject to interference by antibodies against the tag domains when their epitopes are close to each other.

Using the dotLab™ System, we have developed a simple assay to evaluate antibody expression levels, and monitor real-time antibody-antigen binding. A rabbit-anti-mouse Fc (b-RAM-Fc) sensor is used to capture antibodies in hybridoma supernatants. The antigen binding is monitored by attaching antibodies to the specific antigen which is followed by a buffer wash to eliminate antibodies that do not interact with the antigen. Antibodies are then ranked on expression levels and apparent binding affinity.

Figure 2. When a flowing stream of biological sample is introduced into the device's flow channel, target molecules bind to the patterned capture molecules, or antigen spots. An antibody binding to a patterned antibody is a result of the antigen-antibody reaction. The presence of the antigen to the antibody increases the height of the surface pattern, producing an increased phase shift in the reflected laser light, which in turn causes an increase in the diffraction signal intensity.

Figure 3. Each dotLab Sensor contains eight assay spots along a linear flow channel. The dotLab System introduces samples and assay reagents into the dotLab Sensor using an automated sampling system and high-precision fluidic controller. Binding of target molecules to immobilized capture molecules is detected by illuminating the underside of each spot with focused laser light. Changes in the diffraction signal, which is detected using photodiodes, allow the detection beam to pass through the flow channel, providing an ideal platform to work with complex biological samples. A variety of sensor surfaces are available including Axela Protein G, and Goat Anti-Mouse-Fc.

Figure 4. Schematic assay format. Biotinylated RAM-Fc immobilized on an avidin surface captures antibodies in hybridoma supernatants, allowing the evaluation of the antibody amount. Matrix factors are eliminated before specific antigen binding, which can be verified and enhanced by an anti-tag antibody.

Figure 5. Capturing hybridoma supernatant antibodies. Shown below is a real-time data trace of b-RAM-Fc immobilization onto the avidin sensor surface, and subsequent capture of antibodies from an unpurified hybridoma supernatant.

Figure 6. Evaluation of relative amount of antibody. Relative concentrations of supernatant antibodies were determined by normalizing to 1) surface b-RAM-Fc density, and 2) the preference of b-RAM-Fc to mouse IgG subclasses by using known amounts of purified mouse IgG subclasses antibodies (data not shown). The normalized data reveals the relative antibody amount present in the hybridoma supernatants. By capturing the Fc portion of the antibodies, the antibody binding sites are rendered upright for unobstructed access of antigen to the antibodies.

Since the assay uses a polyclonal RAM-Fc as the ligand on the surface, the antibody-antigen interaction occurs between a heterogeneous ligand surface and analyte in solution. Although the binding mechanism involves complex kinet, the simple assay can be used to compare relative antibody reactivity by normalizing the antigen binding to the captured antibody regardless of antibody subclass.

Hybridoma Supernatants

Figure 7. Direct detection of antigen binding. The 37 IdA GST-ASCL1 (10 µg/mL) can be readily detected. The detection is verified by the HRP-conjugated polyclonal rabbit anti-GST (negative control not shown). Reactivity of supernatant antibodies can be compared with the antigen binding alone, antigen followed by an anti-GST antibody, or the antigen preincubated with an anti-GST antibody. The preincubation method provides the advantage of a higher signal-to-noise ratio, and also information about relative epitope distribution (see Figure 9).

Figure 8. Comparison of antigen binding. Binding profiles of the recombinant antigen GST-ASCL1 to hybridoma supernatants 1C5, 3D3, and TE11 shows a comparison of relative antibody-antigen affinity of different antibodies without antibody concentration effects. The signal is normalized to the amount of supernatant antibodies captured by b-RAM-Fc.

Recombinant Antigen Binding to Supernatant Antibodies

Figure 9. Effect of polyclonal anti-GST. By preincubating a polyclonal anti-GST with GST-ASCL1, the signal-to-noise ratio of the antibody-antigen interaction can be increased. When an HRP-conjugated polyclonal rabbit anti-GST (HPR-conjugated GST) is preincubated with the antigen, the signal-to-noise ratio of the three antibodies were consistent with those of antigen alone (compare to Figure 8). However, when an HRP conjugated polyclonal goat anti-GST was used (right panel), the binding profiles were altered, suggesting that at least one or more of the epitopes on the recombinant antigen is not close to the GST tag. Signals were normalized to the amount of supernatant antibodies bound to b-RAM-Fc. This result suggests that the presence of a polyclonal anti-tag antibody might shield the epitope from being recognized by candidate antibodies when used together. Therefore, caution should be exercised when selecting appropriate anti-tag antibodies in assay development.

Antigen Binding Comparison with anti-GST

Antibody Pairing

Figure 10. dotLab™ System for antibody pairing. By using proper assay design and procedure, the system is capable of evaluating antibody pairs that can form sandwich complexes in solution. As an example, a mouse anti-Myo monoclonal Ab 295 was first captured by b-RAM-Fc on the sensor surface (upper panel). The surface was then effectively blocked by Mouse IgG. A pairing anti-Myo monoclonal Ab 104 was added to complete the sandwich formation by recognizing a different epitope on myoglobin. The lower panel shows a no-antigen negative control. Preliminary results with hybridoma supernatants 1C5, 3D3, and TE11 showed no clear sandwich complex formation between each other (data not shown).

Conclusion

The dotLab™ System is a powerful tool for semi-automated characterization of antibodies in crude hybridoma supernatants. In this simple and low cost approach, the relative antibody expression levels can be evaluated using a RAM-Fc capture surface. Relative antibody-antigen antibodies can then be compared by normalizing the binding to the expressed antibody immobilized on the surface. By applying an appropriate assay design, relative epitope/tag distribution and antibody pairing also can be assessed.