Affinity of antibody detection on Countstar® Rigel

1. Introduction

Antibodies, also known as immunoglobulins that is used by immune system to against infiltration of pathogens. Affinity of antibody measured by immunofluorescence is commonly used in selection of biosimilar product in pharmaceutical industry to analyze effectivity of the monoclonal antibody. Currently, quantification of the affinity of antibody is analyzed by flow cytometry. Countstar Rigel also can provide a quick and easy way to evaluate the affinity of antibody.

Countstar® Rigel is an image-based cell analysis platform with fluorescence detection. Countstar Rigel can evaluate antibody affinity base on detecting the average fluorescence intensity of different antigen-antibody reaction by indirect immunofluorescence method.

Figure 1. Countstar® Rigel system combines the functionalities of a digital microscope, an image cytometer and a cell counter in a single bench top instrument.

2. Instruments and Materials

Countstar® Rigel

Countstar® Chamber slides

0.2% Trypan blue solution (CS0101001-50)

DMEM (Hyclone-SH30243.01)

FBS (Hyclone-SH30084.03)

Trypsin (Hyclone-SH30042.01)

002-BMK1, 002-BMK2, AB1, AB2, AB3, AB4, AB5, AB6, AB7, AB8, AB9, PCSK-9 as primary antibodies. (002-BMK1 and 002-BMK2 antibodies are the origin drug that can bind with X protein of CHO cells specifically, and PCSK-9 was a negative control antibody.)

5μg/mL Alexa Fluor® 488 Goat anti-mouse IgG (Biolegend) as secondary antibody.

3. Methods

3.1 Staining Procedure

(1). Added suspension cell medium contained 0.3 million CHO cells into each well of 96 wells plate

(2). Centrifuge 96 wells plate at 400g for 3-5 minutes, remove the medium, and then re-suspend cells in 100μL PBS;

(3). Mix the samples gently and centrifuge 96 wells plate at 400g for 3-5 minutes, remove the medium, and then re-suspend cells in 100μL staining buffer;

(4). Preparing different concentrations((0.01, 0.039, 0.156, 0.625, 2.5, 10μg/mL) of 002-BMK1, 002-BMK2, AB1, AB2, AB3, AB4, AB5, AB6, AB7, AB8, AB9, PCSK-9 as primary antibodies to add to CHO cell line(as figure 2 show).

(5). Incubate for 60min at 37℃ in the dark;

(6).Centrifuge the cell samples at 400g for 3-5 minutes, remove the medium, and then re-suspend cells in 100μL PBS;

(7). Mix the samples gently and centrifuge cell samples at 400g for 3-5 minutes, remove the medium, and then re-suspend cells in 100μLstaining buffer ;

(8). Add secondary antibody (5ug/mL) into each wells;

(9). Incubate for 30min at room temperature (25℃) in the dark;

(10). Repeat Step 6 and Step 7;

(11). Mix the samples, load 20μL of samples into Countstar slide chamber and analyze samples by Countstar® Rigel.

	1	2	3	4	5	6	7	8	9	10	11	12
Antibodies	**002-BMK1**	**002-BMK2**	**AB1**	**AB2**	**AB3**	**AB4**	**AB5**	**AB6**	**AB7**	**AB8**	**AB9**	**PCSK9**
A	10	10	10	10	10	10	10	10	10	10	10	10
B	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
C	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625
D	0.156	0.156	0.156	0.156	0.156	0.156	0.156	0.156	0.156	0.156	0.156	0.156
E	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.039
F	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

μg/mL concentrations

Other wells untapped

Figure 2 Layout of antibodies conditions in 96-well plate. Each well containing CHO

3.2 Imaging and analysis with Countstar® Rigel

(1). Cell number and cell viability was detected by using “Trypan Blue Viability” directly.

(2). Signal-color application procedure was created by setting Green channel to image Alexa Fluor® 488 fluorescence.

(3). 3 fields captured from each chamber.

(4). Data was displayed on the screen directly when testing. After imaging and initial analysis were completed, data were exported and analyzed.

4. Result

4.1 Imaging and analysis with Countstar® Rigel imaging system

The figure 3A suggest that fluorescent signal is getting stronger as the concentration increasing. PCSK-9 was a negative control antibody that would not bind with the CHO cells, while 002-BMK2 antibodies can bind with X protein of CHO cells specifically. Therefore, there has strong fluorescent signal in 002-BMK1 image, but very weak signal in PCSK-9 (As figure 3C and 3D shown)

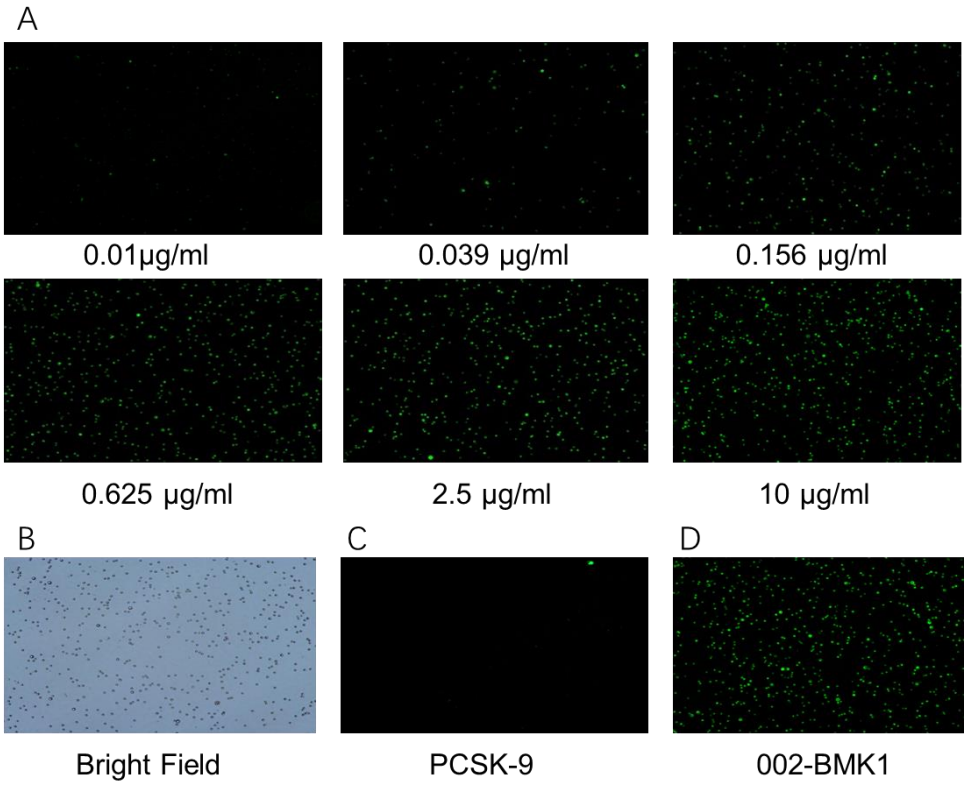
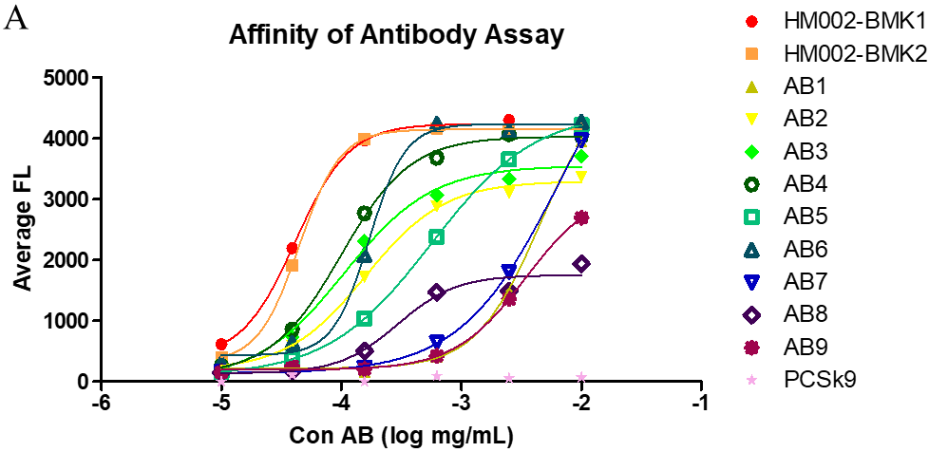


Figure 3 Imaging captured by Countstar Rigel. (A). Fluorescent Images of 0.01, 0.039, 0.156, 0.625, 2.5, 10μg/ml of AB4 treated CHO cells. (B). Bright Field Image of 10μg/ml PCSK-9. (C) Fluorescent Images of 10μg/ml PCSK-9 ;(D) Fluorescent Images of 10μg/ml 002-BMK1

4.2 Quantitative analysis of affinity of antibody

Affinity of antibodies can be quantified by the value of average fluorescence intensity of different antigen-antibody reactions by Countstar Rigel (Fig. 4A). Graphpad Prism 5 was used to evaluate the EC50 of antibodies (Table. 4B). Value of LogEC50 of each antibody suggest that the affinity of original drugs were better than generic drugs. And AB2, AB3, AB4, AB6 have relatively high affinity to the CHO cells compared with other generic drugs (Fig. 4C). The Countstar® Rigel system can evaluate affinity of antibodies directly and reliably.

A



B

	HM002-BMK1	HM002-BMK2	AB1	AB2	AB3	AB4	AB5	AB6	AB7	AB8	AB9	PCSk9
Bottom	388.9	321.8	215.7	187.7	24.26	115	90.4	437	137.9	135.9	194.7	NA
Top	4238	4148	4982	3299	3551	4031	4455	4235	6508	1754	3202	NA
LogEC50	-4.382	-4.351	-2.344	-3.796	-3.975	-4.005	-3.25	-3.769	-2.172	-3.53	-2.468	NA
HillSlope	1.944	2.532	1.629	1.326	1.186	1.49	1	2.884	1.044	1.768	1.475	NA
EC50	0.00004153	0.00004457	0.004533	0.00016	0.00011	9.9E-05	0.000563	0.00017	0.00673	0.0003	0.003405	NA

C

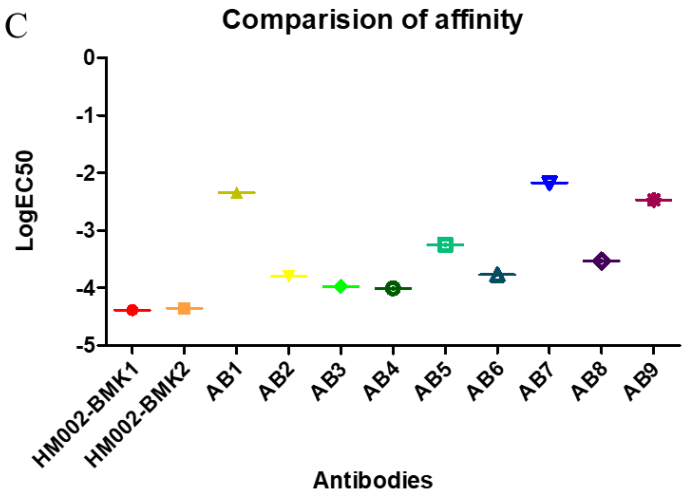


Figure 4 Quantitative analysis results for affinity of antibody. (A) Average fluorescence intensity in different antigen-antibody reactions varied with different concentrations of antibodies; (B) Table: Value of EC50 of different antibody. (C) The comparison of affinity of antibody

5 Conclusion

The Countstar® Rigel system provides a rapid, direct and easy means of evaluating affinity of antibodies. (1) The result clearly show the tendency that the higher concentration of the antibody samples have stronger fluorescent signal. (Fig. 4A). (2) PCSK-9, negative control antibody, barely see the fluorescent signal in images (Fig 3A). (3) And the origin drug show a better affinity than the generic drugs. For affinity of antibody. Countstar Rigel supplies a sophisticated and reliable method.