Optimizing animal-free CloneDetect assay to maximize the sensitivity of real-time local detection of human IgG antibody production using ClonePix system

Paula Zadek, Carola Mancini, Rebecca Kreipke,
Shan Liu I Molecular Devices, LLC

Abstract

How to screen and select clones of interest with high specificity and high efficiency is known as one of the bottlenecks for therapeutic antibody engineering and cell line development. When added to semi-solid media at the moment of plating, recombinant monoclonal CloneDetect K8495 (animal origin-free) diffuses freely, creating immunoprecipitation complexes with target immunoglobulins as they are secreted from the colonies. These complexes generate localized fluorescent signals that, under optimized assay conditions, are proportional to the amount of IgG secreted by a specific clone in the real-time of the IgG quantification conducted. However, due to its monoclonality and high specificities with a single binding site within the human IgG Fc region, if the clones to be screened have low levels of expression of the target IgG molecules, assay optimization is required to achieve optimal results.

In this study, we have explored the usage of recombinant Pro A or Pro G, the two most commonly used proteins for the purification of IgG antibodies in the Biopharma manufacturing process, combined with CloneDetect K8495 to enhance the fluorescent signal for IgG detection/quantification. The results have shown that adding Pro G at the final concentration of 1.0 µg/ml produced the best outcome with over 5-fold fluorescent signal enhancement, compared to the control assay without adding Pro G optimizer. The combination of Pro G and CloneDetect K8495 amplified the fluorescent signal and improved detection, due to the stabilization of the complexes formed by the target protein, Pro G, and CloneDetect reagent. This approach can be applied to a variety of cell line models that express proteins that bind to the stabilized complex formed with CloneDetect and can be imaged, analyzed, and picked by the ClonePix® system (Figure 1).

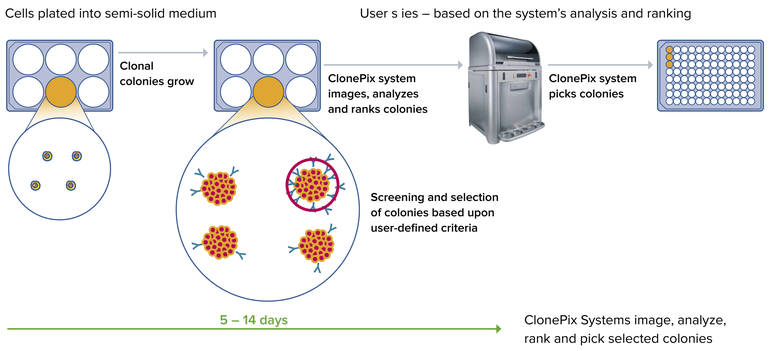

Figure 1. ClonePix Systems image, analyze, rank and pick selected colonies in 1-step process to increase cell line development workflow throughput and process efficiency.

Materials and methods

Prepare Pro A and Pro G stock solutions

Table 1. Add 10 ml of sterile cell culture grade water or DPBS solution to each vial to prepare 0.5 mg/ml stock solution, mix and wait for 10–15 mins in room temperature for complete hydration before filter the stock solution through 0.2µm PES filter and make aliquots. Store aliquots at 4°C for up to 4 weeks or long-term storage at -80°C.

Catalog #	Product Description (MD: Molecular Devices)
K8495	MD Recombinant CloneDetect, Anti-Human IgG (Fc) Specific, Monoclonal, FITC Label, Sterile, Azide-Free, BSA-Free, Animal Origin-Free, Low Endotoxin, 10,000 U / 1ml
K8840	MD CloneMedia CHO Growth A without L-Gln, Semi-Solid Media for CHO Cells, 1 × 90 ml
K8860	MD XP Media CHO Growth A without L-Gln, Liquid Media for CHO Cells, 1x 1000 ml
K8525	MD CloneXL reagent, 5 x 2 ml, 50x concentrated, ACF (animal-component-free) supplement for CHO, HEK293 Single-cell Cloning
21193	Thermo Fisher Pierce™ Recombinant Protein G, 5mg
21184	Thermo Fisher Pierce™ Recombinant Protein A, 5mg
A2916801	Thermo Fisher Gibco 200mM L-Glutamine, 100mL
657185	Greiner Bio-One CELLSTAR Cell Culture 6-well Plates for Suspension Cultures

Materials and methods

Cell culture, plating and imaging analysis

CHO cell line model with low human IgG expression (MD) was maintained in XP Media CHO Growth A with 8mM L-Glutamine and passaged twice a week. 100 µL of log stage cell suspensions at a density of 25,000 – 50,000 cells/ml (final plating density is ~250 – 500 cells per ml) were added to 9.9 mL premixed semi-solid media with the following components:

- 9.0 ml CloneMedia CHO Growth A K8840
- 0.4 ml L-Glutamine
- 0.2 ml CloneXL CHO ACF supplement K8525
- 0.2 ml XP Media CHO Growth A without L-Gln K8860
- 0.1 ml Recombinant CloneDetect K8495

Add different concentration (0.0 µg/ml, 0.5 µg/ml, 1.0 µvg/ml) of recombinant Pro A or Pro G to the mixed media, using the stock solution (0.5 mg/ml) prepared before. The final mixed semi-solid media containing supplements, cells, CloneDetect K8495, and either Pro A or Pro G was plated at 2.0 ml/well into each well of Greiner Bio-One CellStar 6-well plate. Space between wells was filled with sterile water to minimize evaporation. Plates were placed in an incubator with > 85% humidity, 5% CO_2 at 37°C. The incubation time and optimal imaging timepoint will be determined experimentally. In this study, the imaging and analysis on the ClonePix system were performed on Day 13 post plating (Figure 2).

Results

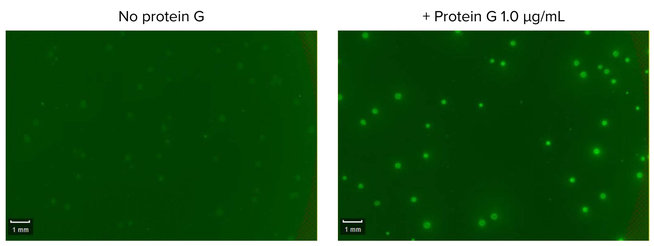

Figure 2. One round of K8495 assay optimization increased the fluorescent signal by over five folds as measured using Sum Total Intensity. Sum Total Intensity is defined as the Sum Fluorescent Intensity of All Pixels of the colony divided by # of Pixels within the colony boundary + halo boundaries with halo determined as 3 x colony diameter (see Appendix A for ClonePix parameters).

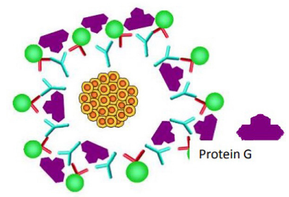

Figure 3. Protein G binds with high affinity to the FC region of IgG molecules. This binding facilitates the formation of the complex among CloneDetect K8495 and the secreted IgG proteins thereby amplifying the signal for enhanced detection.

n=3	Optimizer	Final Conc. (µg/ml)	Mean	± SD	Fold
Control	Pro G	0	73,022	2,296	1.0
	Pro A	0	72,781	3,448	1.0
Group A	Pro G	0.5	173,915	8,579	2.4
	Pro A	0.5	96,944	3,457	1.3
Group B	Pro G	1.0	390,744	15,474	5.4
	Pro A	1.0	165,600	4,931	2.3

Table 2. CloneDetect K8495 was diluted 100 times, together with optimizer Pro G or Pro A at various final concentration (0, 0.5, 1.0 µg/ml) , were added to the semi-solid media with cells on plating date. Each data point represents the mean value of florescence intensity from 3 replicates (n=3), ≥120 colonies measured by ClonePix system on day 13 post plating.

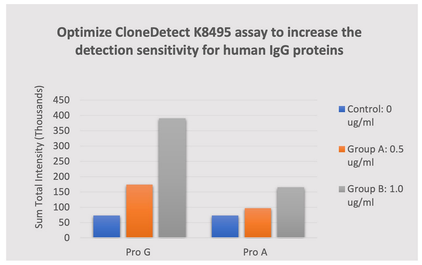

Figure 4. Each date point represents the mean value of Sum Total Intensity (FITC500ms) from 3 replicates. All assays were conducted on the same cell population. Adding Pro G 1.0 µg/ml to K8495 assay produced the optimal outcome at 5.4-fold increase in the fluorescent signal compared to the addition of Pro A at a 2.3-fold signal increase.

Appendix A

ClonePix parameter setting reference

Microplate:	Greiner 6 Well	
Read Barcode:	False	
Barcode Failure:	Auto-Generate	
Source Plate Options:	Prompt on empty	
Processing Algorithm:	EdgeDetect	
Average Colony Diameter:	880µm	
Exterior Statistics Diameter Multiplier:	x3	
Use each colony diameter for exterior statistics:	True	
Discard Groups:	NC Irregular 1	IF Compactness < 0.30
	NC Irregular 2	IF Axis Ratio < 0.30
Groups:	Edge Excluded	IF Edge Excluded = True
	Too Big	IF Total Area > 0.20 mm²
	Too Small	IF Total Area < 0.02 mm²
	Irregular 1	IF Compactness < 0.60
	Irregular 2	IF Axis Ratio < 0.60
	Proximity	IF Proximity < 0.90 mm
	Ungated	Anything else
Optical Configurations:	Description	TransWL
	Emission Filter	WHITELIGHT
	Excitation Filter	WHITELIGHT (TRANS)
	Exposure	100
	LED Intensity	2
	Prime Config	True
	Description	FITC 500 msec
	Emission Filter	EGFP/FITC
	Excitation Filter	EGFP/FITC
	Exposure	500
	LED Intensity	128
	Prime Config	False

Protein G (Pro G) is a large cell surface binding protein that has a repeating 55-residue domain that binds with high affinity to the FC region of IgG[1]. Together with Protein A (Pro A), binding to large amounts of IgG at near physiological pH and ionic strength, these two proteins are extensively used for the purification of IgG antibodies[2] in the Biopharma manufacturing process. Pro G binding can stabilize the complex structure formed between recombinant CloneDetect K8495 and the secreted IgG antibody proteins thereby creating a complex with enhanced detection sensitivity. Here we exploit that property of Pro G versus that of Pro A to increase the fluorescence detection on the ClonePix® System. The ClonePix images and picks mammalian cell colonies based on their productivity of secreted antibodies or cell surface proteins with a variety of measures that include the secreted or surface binding proteins fluorescent signal[3]. Utilizing a combination of imaging and robotics, ClonePix System screens for clonality and productivity simultaneously prior to collecting the highest producing clones in a rapid one-step process.

How to screen and select clones of interest with high specificity and high efficiency is known as one of the bottlenecks for therapeutic antibody engineering and cell line development[4]. There are many factors that will impact the detection sensitivity and clone screening process: 1) low levels of target protein expression 2) misfolded target protein trapped inside the cells 3) low binding affinity of target protein to detection reagent 4) unstable bindings of target protein to detection reagents.

The combination of Pro G and CloneDetect K8495 amplified the fluorescent signal and improved detection, due to the stabilization of the complexes formed by the target protein, Pro G and CloneDetect reagent. This approach can be applied to a variety of cell line models that express proteins that bind to the stabilized complex formed with CloneDetect, and can be imaged, analyzed and picked by the ClonePix system.

Key Benefits

- Over 5-fold of signal improvement with 1 round of assay optimization
- Real-time monitoring therapeutic antibody production in local cell culture environment
- Easy setup and applicable to other cell models and proteins quantification
- 100% hands-free and walk-away solution to complement ClonePix 1-step workflow and improve clone screen efficiency

References

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