Accelerating the Antibody Discovery Process

Multiplexed Analysis Using the Mouse IgG Type and Titer Kit

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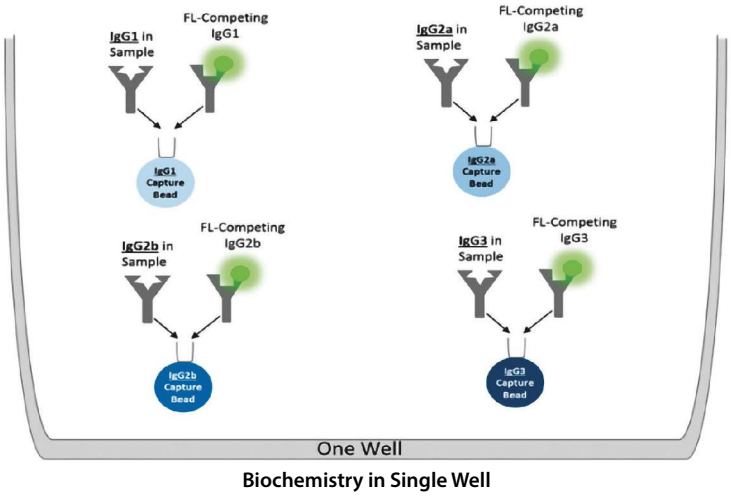

Figure 1. Competition assay schematic

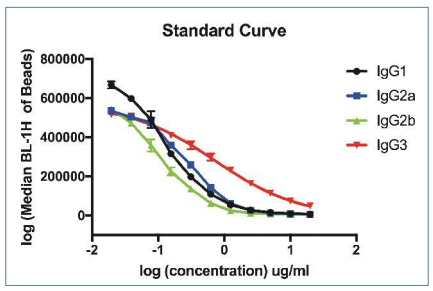

Figure 2. IgG isotype standard curves

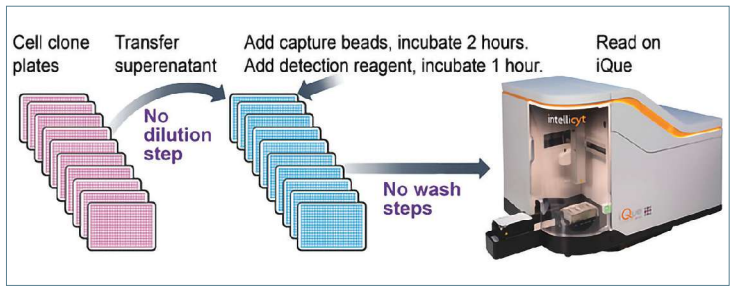

Figure 3. Workflow of the type and titer kit

Immunotherapy is a rapidly growing field with multiple therapeutic modalities including, but not limited to, antibody drug conjugates, bispecific antibodies, and CAR-(chimeric antigen receptor) containing T cells. The unifying component of all these modalities is an antibody or antibody fragment and as of 2016 over 60 antibody-based drugs have been approved for therapeutic use with global revenues of over $89 billion.[1] Antibody discovery is, however, still a challenge and methods to increase the efficiency and throughput of the screening process is sorely needed.

The Intellicyt® iQue Screener Plus platform provides solutions at several points along the discovery process. Currently we use the iQue® Screener Plus in our protein production pipeline where its high-throughput flow cytometry capabilities aid in quality control of our mammalian cell culture process. Recently, Intellicyt released their Cy-Clone™ PLUS kit that aids in rapid clone selection of monoclonal antibody (mAb) producer lines by simultaneously detecting and quantitating human Ig production and determining cell viability. Here we describe an alternate version of this system to be used further upstream in quantifying and isotyping Ig producing hybridomas generated from mouse immunization called the Intellicyt Mouse IgG Type and Titer Assay Kit.

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Currently our antibody discovery workflow involves the fusion of murine B cells from immunized mice with a myeloma line for the generation of hybridomas. The hybridoma cells are initially plated in oligoclonal pools that are then screened for antigen positivity. The Mouse IgG Type and Titer Assay Kit is used at this step to quantify and isotype the hybridomas which then allows us to rapidly select the most productive clones that correlate with antigen positivity. The Mouse IgG Type and Titer Assay Kit is then used again at the monoclonal stage to inform the selection of our final clones.

The Intellicyt Mouse IgG Type and Titer Assay Kit allows for multiplexed analysis of four isotypes of mouse IgG (IgG1, IgG2a, IgG2b, IgG3) by combining four isotype-specific capture beads in a single bead cocktail *(Figure 1)*. Multiplexing greatly streamlines the quantification and isotyping process. Comparatively, four enzyme-linked immunosorbent assays (ELISA) would be required to complete the same analysis. FITC-labelled IgG antibody of each isotype is added to compete with the IgG produced by the hybridoma for the capture beads. In this manner, the secreted IgG concentrations are inversely proportional to the FITC fluorescence intensity of each isotype-specific bead. Utilizing the Intellicyt Mouse IgG Type and Titer Assay Kit IgG standards, a 12-point standard curve to quantify the antibody content produced by each hybridoma is prepared *(Figure 2)*. Next, hybridoma supernatants are directly assayed in 96-well format using the high sensitivity protocol to help ensure all antibody-producing hybridomas are identified. Briefly, 20 microliters of hybridoma supernatant is pre-incubated with the capture bead cocktail for 2 hours. FITC-labelled IgG antibody is then added and incubated for one hour. The standards and hybridoma samples can then be directly analyzed on the iQue Screener Plus without a wash step *(Figure 3)*.

Utilizing the standard curves assayed under the same conditions in a separate assay plate, the data from hybridoma supernatants can be analyzed using the ForeCyt® software to analyze the quantity and isotype(s) of the antibody(ies) being produced in each well *(Figure 4)*. By setting concentration thresholds of each isotype present in each well, we can identify hybridomas which predominantly produce a single isotype of IgG *(Figure 5)*. The ability to identify productive hybridomas and determine each hybridoma's isotype profile helps to inform us of the lead candidates for antigen specificity characterization and hybridoma sub-cloning early in the antibody discovery process. The isotype information also streamlines our sequencing pipeline as we're able to use isotype-specific primers rather than pooled oligos as is done when the isotype is unknown.

Summary

The Intellicyt Mouse IgG Type and Titer Assay Kit allows for direct analysis of hybridoma supernatants in a multiplexed format, saving valuable time and resources compared to the traditional ELISA process. The low detection limit of the high-sensitivity procedure, capable of detecting antibody concentrations as low as 0.05µg/mL, makes the kit also applicable to single B cell-based antibody screening campaigns which typically produce much lower concentrations of IgG.[2] The ability to determine the isotype of the antibodies generated in either hybridomas or in single B cell sorts greatly facilitates the sequencing of discovered antibodies. The data represents assays prepared in a 96-well format, but the assay kit can be scaled and analyzed in a 384-well format for more rapid antibody discovery. GEN

References

1. Carter, P. J. & Lazar, G. A. Next generation antibody drugs: Pursuit of the 'high-hanging fruit'. *Nat. Rev. Drug Discov.* 17, 197–223 (2018).

2. Kuraoka, M. et al. Complex Antigens Drive Permissive Clonal Selection in Germinal Centers. *Immunity* 44, 542–552 (2016).

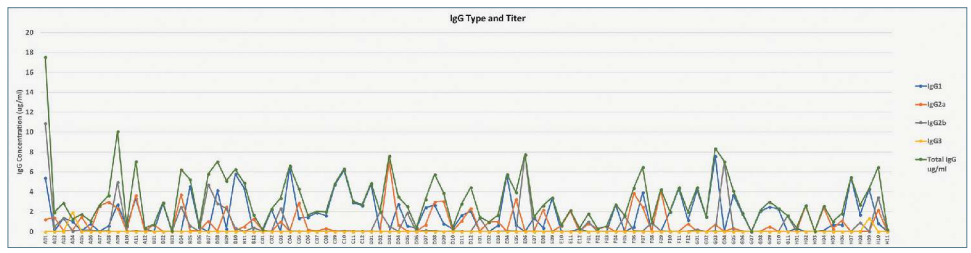

Figure 4. Analysis of hybridoma supernatants

Figure 5. Determination of isotype dominance using profile maps