Building a better bioassay: frozen, thaw-and-use cells key to reduced variability ADCC bioassay

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Although conventional ADCC cytotoxicity assays are ADCC mechanism of action (MOA)-based, they universally suffer from complex protocols, are tedious to perform and exhibit high variability due to heterogeneity of NK donors. Promega recently introduced a novel bioluminescent assay for the quantification of Fc effector function, as a suitable replacement for such classic ADCC assays.

Antibody-dependent cell-mediated cytotoxicity (ADCC) is the main MOA of antibodies through which virus-infected or other diseased cells are targeted for destruction by components of the cell-mediated immune system. Measurement of Fc effector function, primarily as a measure of ADCC activity for antibody-based molecules, is a critical functional assay in the development and manufacture of biologics drugs.

The industry has needed better cell-based assays that can be used as bioassays to demonstrate functionality of monoclonal antibodies, and other protein-based drugs, with consistent performance (eg low variability and high reproducibility). The Promega ADCC Reporter Bioassay was developed to meet such a need.

The commercial assay is ADCC MOA-based and features frozen, thaw-and-use effectors cells, as well as optimised reagents and detailed protocol to perform a reporter-based ADCC bioassay in a single day. The ADCC Reporter Bioassay correlates with classic cytotoxic ADCC assays and is a suitable replacement for such cumbersome and highly variable assays.

How the novel bioassay works

The ADCC Reporter Bioassay uses an alternative readout at an earlier point in ADCC MOA pathway activation: the activation of gene transcription through the NFAT (nuclear factor of activated T-cells) pathway in the effector cell. In addition, the engineered Jurkat cells stably express the Fc RIIIa receptor, V158 (high affinity) variant, and an NFAT response element driving expression of firefly luciferase resulting in an easy-to-read light output. The Promega reporter-based ADCC bioassay, which can be performed in a single day (or overnight) is shown in **Figure 1**.

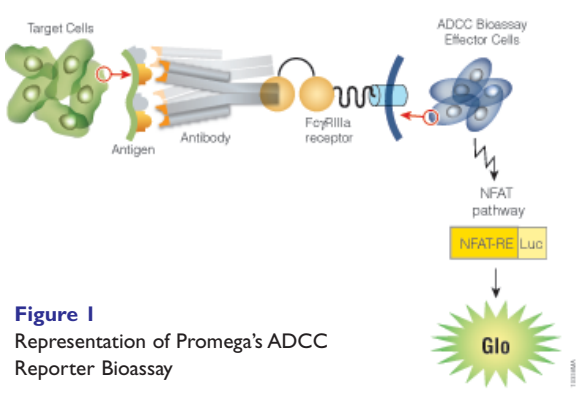

Figure 1
Representation of Promega's ADCC Reporter Bioassay

The bioassay shows good linear correlation between levels of glycosylation or afucosylation and ADCC activity. All of these features indicate the assay is suitable for use across biologics drug development programmes including as a lot release potency bioassay. Quantifying the biological activity of any therapeutic antibody is an important step in the development and manufacture of biologics.

Cells as reagents

In the novel ADCC Reporter Bioassay, the effector cells can be considered as assay reagents. The frozen, thaw-and-use Jurkat effector cells are prepared under stringently controlled conditions and are used immediately upon thawing without further culture. By formatting the effector cells as reagents, the new reporter-based bioassay outperforms classic ADCC in many key parameters: low variability, improved accuracy and precision, ease of assay procedure and low background (sensitivity).

Promega's reporter-based ADCC bioassay has undergone extensive testing both in-house and in the hands of global collaborators in the biologics industry. As an indicator of its applicability, it is now being used by manufacturers of biologics as well as contract research organisations.

Summary

The novel, bioluminescent reporter-based ADCC bioassay recently introduced by Promega possesses low variability, is simple-to-use, and is configured in a convenient format to quantify the Fc effector function of antibodies in ADCC. A key feature is the frozen, thaw-and-use format of the effector cells with prime benefit being the low variability. The bioassay is widely applicable to antibody drug candidates being developed as cancer therapeutics, and has demonstrated good precision and accuracy in bioassay qualification studies.

Web: www.promega.com/drug-discovery

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