CLARIOstar and A combination for proven success

HTRF and BMG LABTECH microplate readers: A combination for proven success

Carl Peters, BMG LABTECH, Cary, NC

Introduction

BMG LABTECH and Cisbio have a long history of collaboration, starting with the RUBYstar, a reader dedicated to detection of HTRF technology. Today, BMG LABTECH continues to make it a priority that our HTRF detection is the best in the industry. From our Gold Standard PHERAstar FS HTS reader to our assay development and core screening platform the CLARIOstar, BMG LABTECH readers can make HTRF assays perform their best. Due to their exceptional sensitivity we are proud to say that both of these readers are certified to perform HTRF in white and black plates. The result of our collaboration with Cisbio has been nine application notes on a variety of topics. Here, we show a sample of the applications from which our microplate readers have been used to detect HTRF.

AN 154: Cortisol Assay Performed on the PHERAstar

One of the first application notes in the BMG LABTECH/Cisbio collaboration. This note used the original PHERAstar plate reader to detect cortisol as a means to determine 11 β-hydroxysteroid dehydrogenase type 1 activity, a key enzyme in everyday metabolism.

Fig. 3: Cortisol Assay Principle. In the absence of cortisol the anti-cortisol will bind to cortisol antibody and a signal HTRF signal will be seen. Increasing cortisol displaces the cortisol 42 resulting in decreased HTRF signal.

The note used a cortisol titration curve to show that nearly identical results were seen if the detection step was incubated for 2 or 16 hours. In addition, IC50 values for two known inhibitors were determined using a biochemical assay and the results were found to be comparable to those reported in the literature.

AN 209: GPCR Activation Measured with cAMP and IP-One Cell-based Assays Using PHERAstar®Plus

For HTS the ability to detect inhibitors of GPCRs remains one of the most important tools. This application demonstrates the ability of the PHERAstar®Plus to monitor the 2 major second messengers of this system using the HT-Plex assay.

Fig. 4: IP-One and cAMP HT-Plex assay can be used to monitor GPCR activity.

The app note also notes that this was a very robust assay, based on the calculated 2 prime which was 0.75.

AN 222: PHERAstar FS Discoveres Low Affinity Inhibitors of IP Signaling Pathway

This note again points out the importance of analyzing GPCRs. In this case the assay focused on inositol phosphate signaling an effect of G-coupled GPCRs which leads to calcium release.

Fig. 6: The IP-One assay uses a monoclonal antibody that specifically recognizes IP-1 and is based on a competition format where increased IP-1 displaces labeled IP-1.

The PHERAstar FS results were compared to a HTS device equipped with a CCD camera. The FS exhibited superior assay performance based on Z’, Delta F% and assay window as well as being twice as fast as the CCD-based reader.

AN 279: Methyltransferase Assay that Detects Histone Modifying Activity

The assay kits that Cisbio makes available are constantly being updated to meet the needs of end users. A good case in point is the need for assays to assess the function of enzymes involved in epigenetics. In this application note the Epigenetic Methyltransferase kit was used to detect the activity of the SET 7/9 enzyme which has been identified as a modulator of p30 activity in human cancer cells.

Fig. 8: Epigenetic Methyltransferase Assay Kit Detection Signal. If no SAM was converted from SAM during the enzymatic reaction, SAM will bind to SAMR, leading to a large HTRF signal. The presence of converted SAM will compete with SAMR for binding sites on the antibody resulting in decreased HTRF signal.

The assay worked very well as indicated by the assay quality with all conditions exhibiting a Z’ > 0.50 and EOB of 0.1 as well.

Conclusion

If your goal in HTS the PHERAstar FS is the clear choice, with dedicated laser excitation, optic modules and PMT’s. If you are looking for a reader for assay development you can’t go wrong with the CLARIOstar which is also excellent in HTRF detection. We anticipate continued success in our collaborations with Cisbio Cisbio continues to advance HTTRF technology by developing new targets for their assay. BMG LABTECH will continue to strive to produce microplate readers which are the best at detecting HTRF.

Visit us at www.bmglabtech.com