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Measuring Caspase-3/7 Activity in Serum Using a Luminescent Assay

Seidel, N. *et al.* (2005) The extent of liver steatosis in chronic Hepatitis C Virus infection is mirrored by caspase activity in serum. *Hepatology*. **42**, 113–20.

Invasive liver biopsies are a necessary part of assessing progression of chronic hepatitis C virus (HCV) infection; colorimetric and fluorometric substrate assays are employed to detect caspase activity in cell lysates. Clinical observation of the progression of HCV is not currently possible in serum due to the quenching activity of hemoglobin in the standard colorimetric and fluorometric assays. However, using a luminescent assay avoids the problem of quenching and also provides higher sensitivity. To determine the importance of apoptosis in hepatic C virus (HCV)-associated steatosis, two novel caspase assays were used to detect hepatic caspase activation in sera from HCV patients with different grades of steatosis. The first assay included use of the M30-Apoptosense ELISA kit (Peviva) to evaluate the amount of neopeptide produced by caspase cleavage of cytokeratin-18 (CK-18), an abundant protein in hepatocytes. The M30 antibody used specifically labels only the early apoptotic, not necrotic, cells and immunoprecipitates cleaved CK-18 in HCV serum samples. The second assay used the Caspase-Glo® 3/7

Assay^(a-c), which sensitively monitors active caspase-3 and -7 using a proluminescent substrate. This substrate has a caspase cleavage sequence DEVD in an optimized reagent for both caspase and luciferase activity. Upon cleavage of DEVD by caspase-3 or -7, aminoluciferin release in the presence of luciferase produces light, which is then measured in relative light units (RLU). This study compared sera from patients with chronic HCV infection with sera from healthy controls. Sera were diluted 1:1 in 50 mM/L Tris-HCl (pH 7.4), 10 mM/L KCl and 5% glycerol. A volume of 10 µl of the diluted samples was incubated with an equal volume of the Caspase-Glo® Reagent for 3 hours at room temperature, and luminescence was measured. The application of this Caspase-Glo® Assay in serum allows researchers to monitor caspase activation in easily accessed samples such as serum.

Related Products

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay	10 ml	G8091

For Laboratory Use. Additional sizes available.

^(a)U.S. Pat. No. 7,148,030 and other patents pending.

^(b)U.S. Pat. Nos. 6,602,677 and 7,241,584, Australian Pat. Nos. 754312, 785294 and other patents and patents pending.

^(c)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

Caspase-Glo is a registered trademark of Promega Corporation.

Measuring NFκB Activity Using Two-Color, Dual-Luciferase Reporters

Davis, R.E. *et al.* (2007) A cell-based assay for IκBα stabilization using a two-color dual luciferase-based sensor. *ASSAY Drug Dev. Technol.* **5**, 85–103.

In recent years, one of the major routes to drug discovery and development has been to find and target small molecules that inhibit cell signaling pathways using high-throughput screening (HTS), which increases the odds of quickly discovering significant players in the cell signaling pathways. However, the success of HTS depends on the design and execution of the assays. One essential aspect of such HTS assays is the ability to correct for variation in cell number and viability, while also monitoring modulation of the pathway of interest. Current methods in which coreporters are derived from both firefly luciferase and *Renilla* luciferase allow for correction during data collection, but because two separate reagents are needed, the assay is not ideally suited for HTS. To address these concerns, the authors adapted the Chroma-Luc™ technology for their IκBα-luciferase reporter assay, and they demonstrated several advantages over traditional screening.

The green beetle luciferase (CBG68) was fused to IκBα, allowing measurement of modulations and proteasomal activity, whereas the red beetle luciferase (CBR) was expressed alone to

concurrently measure nonspecific effects and correct for cell number and viability. Because the Chroma-Glo™ Assay measures two luciferases derived from the same parent luciferase, it requires only a one-step substrate addition directly into cultured cells with the activities measured specifically for each luciferase based on the color of emitted light. Therefore, the Chroma-Glo™ Assay is advantageous for HTS. Also, when luciferases with similar amino acid sequences are used, both proteins will presumably respond similarly to nonspecific effects, increasing assay sensitivity. Finally, using an inducible promoter system for both reporters, the responsiveness to inhibitors greatly increased, enhancing throughput and sensitivity over current luciferase assays. All of these advantages allowed miniaturization of the assay to a 1536-well plate for HTS.

Related Products

Product	Size	Cat.#
pCBR-Control Vector	20 µg	E1421
pCBG68-Basic Vector	20 µg	E1431
Chroma-Glo™ Luciferase Assay System	10 ml	E4910

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

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