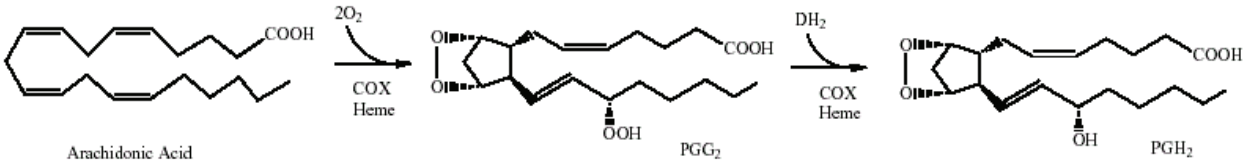
COX flash luminescence screening

Abstract

Cyclooxygenase (COX, also known as Prostaglandin G/H synthase) is a membrane bound enzyme responsible for the oxidation of arachidonic acid to Prostaglandin G2 (PGG2) and the subsequent reduction of PGG2 to PGH2. The conversion is shown as below. These reactions are the first steps in the formation of a variety of prostanoids. COX has been shown to be expressed in at least two different isoforms, a constitutively expressed form, COX-I, and an inducible form, COX-II. COX-I is thought to regulate a number of 'housekeeping' functions, such as vascular hemostasis, renal blood flow, and maintenance of glomerular function. Two cyclooxygenase isozymes, COX-1 and -2, are known to catalyze the rate-limiting step of prostaglandin synthesis and are the targets of nonsteroidal anti-inflammatory drugs (NSAID's). Inflammation mediators such as growth factors, cytokines and endotoxin induce COX-II expression in a number of cellular systems. The effect of various NSAID's on the activity of COX-I and -II is an area of considerable interest. Some methods to determine COX activity involve procedures such as measuring uptake of oxygen using an oxygraph, measuring the conversion of radioactive arachidonic acid, or measuring the prostaglandins formed from PGH2 (such as determining PGE2 using immunoassays6). Most of these methods are complex, time consuming, and are prone to interferences.

The Cyclooxygenase Reaction



COX enzyme+Hemo+Substrate+Arachidonic AcidFlash luminescence

Materials and Methods

The Cayman or Assay Designs' Cyclooxygenase Activity Kit uses a specific chemiluminescent substrate to detect the peroxidative activity of COX enzymes. After inhibition by NSAID's the direct residual activity of COX is measured by addition of a proprietary luminescent substrate and arachidonic acid. Light emission starts immediately and is directly proportional to the COX activity in the sample. The chemiluminescent signal is measured over 5 seconds.

FDSS for Flash Luminescence

FDSS can be used as a versatile tool for not only fluorescent assay but also luminescent assay in both 96- and 384-well microplates.The luminescent signal detection is accomplished using 2 dimensional photon counting detector even in single photon light level. Because of the single photon light level sensitivity and no time lag form dispensing to light detection, FDSS is the most suitable HTS system for flash COX luminescent assay. Here we have validated the COX flash luminescent kit for substrate and arachidonic acid injection into 96- and 384-well plates.

Results and Discussion

The sensitivity of the FDSS was similar to that of PMT based luminometers (Top Counts) for flash COX assays, and allows efficient screening in both 96- and 384-well plates. The FDSS shows good linear response to change in COX concentration and therefore provides a suitable method for studying COX inhibitor in high throughput screening assays. Here we present the stable data for the use of COX in functional HTS with the FDSS, a new generation ultrasensitive luminometer equipped with an integrated pipetting system suitable for HTS flash luminescence.

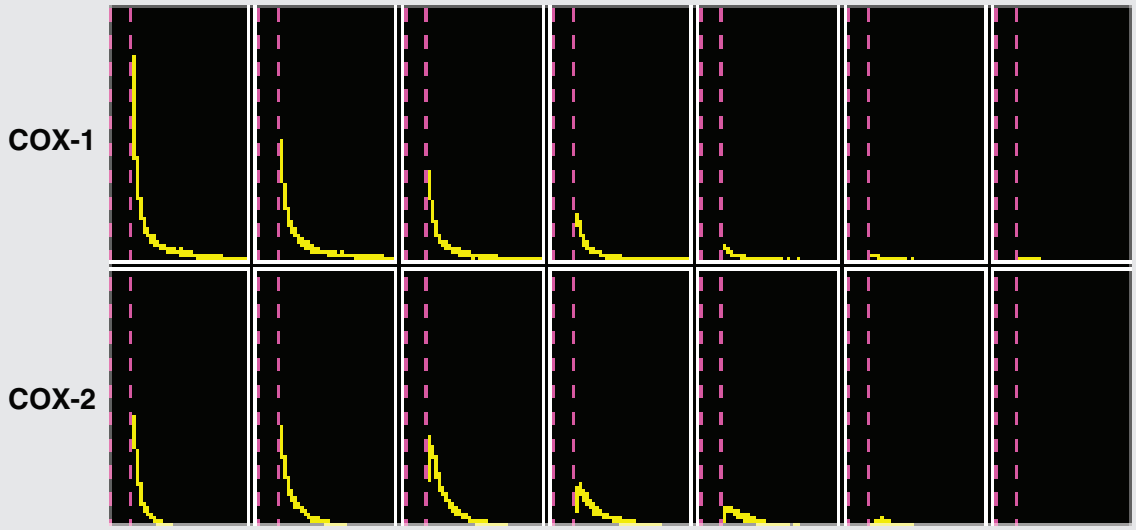


Fig. 1: Dose-response curve of COX-1 and COX-2 activity in 96well.

Consumable

Cayman
Chemiluminescent COX (ovine) Inhibitor Screening Assay
Catalog No. 760101

Sigma
Indomathacin Catalog No. I7378

References

1. Le Poul E, Hisada S, Mizuguchi Y, Dupriez VJ, Burgeon E, Detheux M. Adaptation of aequorin functional assay to high throughputscreening. J Biomol Screen. 2002 Feb;7(1):57-65.
2. Forghani F, Ouellet M, KeenS, Percival MD, Tagari P. Analysis of prostaglandin G/H synthase-2 inhibition using peroxidase-induced luminol luminescence. Anal Biochem 1998 Nov 15;264(2):216-21
3. Xie, W., Chipman, J.G., Robertson, D.L., et al. Expression of amitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc. Nat.Acad. Sci. USA 88, 2692-2696 (1991). ;30(32):7879-83

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