

AlphaScreen Functional cGMP Assay

Sensitive, flexible and cost efficient

The AlphaScreen™ cGMP assay is a homogeneous non-radioactive bead-based system designed to measure cGMP either in cell lysates or in cell-free biochemical assays. The assay measures the activity of therapeutic targets such as phosphodiesterases, guanylate cyclases or nitric oxide synthetases. The assay is based on the competition between the AlphaScreen Biotinylated cGMP Supplement and free cGMP which compete for a limited number of binding sites on cGMP-specific polyclonal antibodies captured on AlphaScreen Protein A-coated acceptor beads.

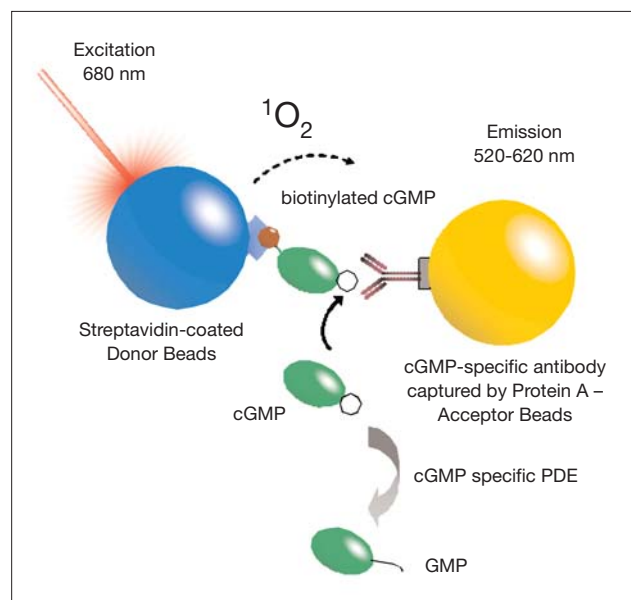
PerkinElmer's AlphaScreen cGMP offers maximum sensitivity and flexibility combined with cost efficiency.

Superior performance and ultra-high throughput screening convenience

- **High sensitivity** – fmol detection
- **High precision** – $Z' > 0.8$ for PDE assays
- **Flexible sample formats** – cell-free or lysed cell formats
- **Easy to automate** – only two steps of addition following cell stimulation or enzymatic reaction
- **Ultra HTS-compatible** – easy to adapt to 96-, 384- or 1536-well plates formats
- **Stable signal** (>20 hours at room temperature) – ideal for batch HTS campaigns
- **Cost-efficient profit from easy miniaturization and precision**

- **Read on any AlphaScreen readers**, such as PerkinElmer's the AlphaQuest®-HTS, the Fusion-Alpha™ and EnVision™ Microplate Analyzers

Assay principle



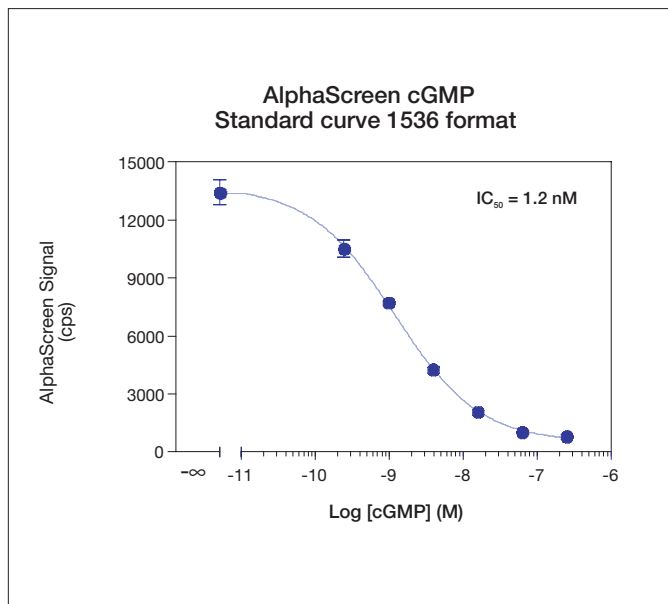
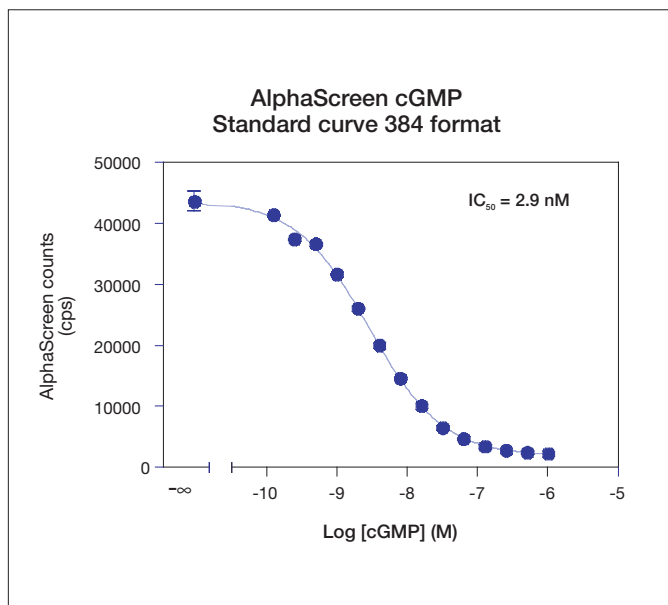
AlphaScreen cGMP Assay Principle. The assay is based on the binding of the AlphaScreen Biotinylated cGMP Supplement (biotin-cGMP tracer) to a specific anti-cGMP antibody. The AlphaScreen Biotinylated cGMP Supplement and the antibody are respectively captured by the AlphaScreen streptavidin-Donor and

Feature	Benefit
Higher sensitivity	Increased data quality
High precision	More robust results
Easy to automate	Increased throughput; higher Z'
Stable signal	Ideal for large screens ensuring high Z'
Low autofluorescence	Lower compound interference
Scalable for 96-, 384-, 1536-well formats	Permits ease of transfer from AD to HTS

Protein A-conjugated Acceptor beads. The high-affinity interaction between the AlphaScreen Biotinylated cGMP supplement and the antibody allows the two types of beads to come into close proximity, resulting in an intense AlphaScreen signal. Unlabelled cGMP competes against the biotinylated cGMP tracer on the antibody binding sites resulting in a concentration-dependent signal decrease.

The graphs below present typical AlphaScreen cGMP standard curves obtained on an EnVision™ instrument in both 384 and 1536 formats (25 µL and 7.5 µL total assay volumes, respectively).

AlphaScreen cGMP standard curves



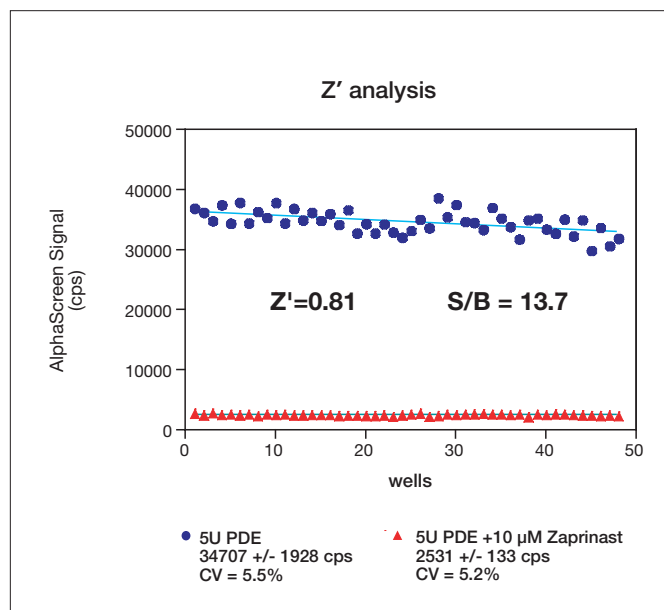
AlphaScreen cGMP PDE assay

The following graph presents the robustness of an AlphaScreen cGMP Phosphodiesterase assay developed with a purified recombinant PDE Type V enzyme, purchased from Calbiochem (Cat # 524715). The Z' study was manually conducted using the 384 format. Two populations of 48 replicates were generated in the presence or absence of 10 µM Zaprinast inhibitor (generic cGMP PDE inhibitor) diluted in 5% DMSO.

Z' values were calculated according to the formula of Zhang *et al.* (JBS, 4:67, 1999). A Z' value of 0.81 and a S/B ratio of 13.7 were calculated from the two populations of data obtained with manual dispensing, indicating that the assay is robust and suitable for compound screening and HTS campaigns.

The AlphaScreen cGMP guanylate cyclase (GC) or phosphodiesterase (PDE) assays require the AlphaScreen Biotinylated cGMP Supplement with antibody (Cat # 6760308 M / R) to be used with existing AlphaScreen Protein A kits (Cat # 6760617C/M/R).

Representative Z' graph for a 384 manual AlphaScreen cGMP PDE assay (PDE Type V).



Ordering information

AlphaScreen cGMP Supplement with Antibody Supplement contains specific anti-cGMP antibody (rabbit polyclonal)	6760308M	0.25 nmol	10,000 assay points
	6760308R	1.25 nmol	50,000 assay points
AlphaScreen Biotinylated cGMP Supplement	6760306M	0.25 nmol	10,000 assay points
	6760306R	1.25 nmol	50,000 assay points
AlphaScreen Protein A Kit	6760617C		500 assay points
	6760617M		10,000 assay points
	6760617R		50,000 assay points

Complimentary products

Microplates	OptiPlates™		
	96 well	6005290	
6005299			200/box
384 well	6007290		50/box
	6007299		200/box
1536 well	6006280		50/box
	ProxiPlates™		
384 well	6006280		50/box
TopSeal™	TopSeal-A™, 96 well	6005185	100/box
	TopSeal-A, 384 well	6005250	100/box

Shift to **better performance** for
your **cGMP functional screens**

Try PerkinElmer's AlphaScreen Biotinylated cGMP Supplement.

For details, visit www.perkinelmer.com/las or call 1-800-762-4000.

**PerkinElmer Life and
Analytical Sciences**
710 Bridgeport Avenue
Shelton, CT 06484-4794 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com



For a complete listing of our global offices, visit www.perkinelmer.com/lasoffices

©2006 PerkinElmer, Inc. All rights reserved. The PerkinElmer logo and design are registered trademarks of PerkinElmer, Inc. AlphaScreen, EnVision, Fusion-Alpha, OptiPlates, ProxiPlates, TopSeal and TopSeal-A are trademarks and AlphaQuest and PerkinElmer are registered trademarks of PerkinElmer, Inc. or its subsidiaries, in the United States and other countries. All other trademarks not owned by PerkinElmer, Inc. or its subsidiaries that are depicted herein are the property of their respective owners. PerkinElmer reserves the right to change this document at any time without notice and disclaims liability for editorial, pictorial or typographical errors.