

Microplates and Microplate Analyzers

EnVision™, Fusion™-α and AlphaQuest®-HTS microplate analyzers and Optiplate™ microplates are optimized to measure AlphaScreen signal and are available from PerkinElmer Life and Analytical Sciences.

For a complete listing of Alphascreen products, please visit:

www.perkinelmer.com/alphascreen

CONTACT PERKINELMER:
www.perkinelmer.com

For all technical assistance or to place an order, please contact:

PerkinElmer Life and Analytical Sciences
710 Bridgeport Avenue
Shelton, CT 06484
USA
800-762-4000 or 203-925-4602
techsupport@perkinelmer.com

European Headquarters
PerkinElmer Life and Analytical Sciences .
Imperiastraat 8
BE-1930 Zaventem
Belgium
Techsupport.europe@perkinelmer.com

Country	Telephone
Austria	0800 293 515
Belgium	0800 94 540
Denmark	80 88 3477
France	0800 90 77 62
Germany	0800 1 81 00 32
Italy	800 79 03 10
Netherlands	0800 02 23 042
Norway	800 11 947
Spain	900 973 255
Sweden	020 79 07 35
Switzerland	0800 55 50 27
United Kingdom	0800 89 60 46

AlphaScreen™ Biotinylated cGMP Supplement with Anti- cGMP Antibody

Product Information & cGMP Standard Curve Protocol

TDS-6760308M-02

AlphaScreen™, AlphaQuest™, EnVision™, Fusion™, OptiPlate™ and TopSeal™ are trademarks of PerkinElmer, Inc.

Milli-Q® is a registered trademark of Millipore Corporation.

LIMITED WARRANTY PerkinElmer BioSignal Inc., a subsidiary of PerkinElmer Life and Analytical Sciences, warrants that, at the time of shipment, the products sold by it are free from defects in material and workmanship and conform to specifications that accompany the product. PerkinElmer BioSignal Inc. makes no other warranty, express or implied with respect to the products, including any warranty of merchantability or fitness for any particular purpose. Notification of any breach of warranty must be made within 30 days of receipt unless provided in writing by PerkinElmer BioSignal Inc. No claim shall be honored if the customer fails to notify PerkinElmer BioSignal Inc. within the period specified. The sole and exclusive remedy of the customer for any liability of PerkinElmer BioSignal Inc. of any kind including liability based upon warranty (express or implied whether contained herein or elsewhere), strict liability contract or otherwise is limited to the replacement of the goods or the refunds of the invoice price of goods. PerkinElmer BioSignal Inc. shall not in any case be liable for special, incidental or consequential damages of any kind.



Product Information

AlphaScreen™ Biotinylated cGMP Supplement with Anti-cGMP Antibody

Catalog No.: **6760308M**
Kit size: **0.25 nmol***
(10,000 assay points)

* This amount of material corresponds to 10,000 assay points when used at the recommended final concentration of 1 nM in a 25 µL reaction

Product

Biotinylated cGMP

1 tube containing 200 µL biotinylated cGMP at 1.25 µM (0.25 nmol) in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. Proclin-300 at 0.05% is added as a preservative. **Store at -20°C.**

Anti-cGMP Antibody

1 tube containing 50 µL of Anti-cGMP antibody in 50% PBS, 50% Glycerol, 1% BSA. Proclin-300 at 0.05% is added as a preservative. **Store at -20°C.**

To perform AlphaScreen cGMP assays, this product is required to be used with the following reagents:

- AlphaScreen Protein A kit
- cGMP competitor

Please see Section 'Materials Required' for more information.

Product QC/QA

AlphaScreen cGMP Supplement lot-to-lot consistency is confirmed by a titration assay to meet defined specifications under standard conditions.

The concentration of the supplement is determined by measuring the absorbance at 252 nm.

Note: The cGMP standard curve protocol provides a means to generate cGMP standard curves and is not representative of an enzymatic assay (cGMP guanylate cyclase, phosphodiesterase or nitric oxide synthase assays). To perform such assays with the AlphaScreen cGMP Supplement, please refer to the appropriate application notes.

cGMP Standard Curve Protocol

Materials required:

AlphaScreen Protein A Kit (PerkinElmer cat No. 6760617)

cGMP competitor: The unlabeled cGMP competitor used for QC purposes is obtained from Sigma (cat No. G6129). Other sources can be used as well.

Reagent Preparation:

Assay Buffer (25 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% Tween 20).

Prepare 50 mL of buffer and adjust pH to 7.4 with NaOH.

Please note that it is strongly recommended **NOT** to use the control buffer from the AlphaScreen Protein A kit.

cGMP competitor

First prepare a 50 µM solution in Assay Buffer. Then prepare a dilution series from 0.5 nM to 5 µM in Assay Buffer. Include a buffer only control.

AlphaScreen biotinylated cGMP Supplement

Dilute the stock solution (1.25 µM) to 5 nM in Assay Buffer.

Acceptor Beads Mix (anti-cGMP antibody / Protein A conjugated Acceptor beads)

Dilute the antibody 20-fold in assay buffer. The anti-cGMP antibody is used at a final dilution of 1:5,000. In 980 µL of Assay Buffer, add 10 µL of the 1:20 antibody solution. Mix gently.

Add 10 µL of the AlphaScreen Protein A coated Acceptor beads at 5 mg/mL to this mixture. Mix gently and pre-incubate the detection mix for 30 minutes at room temperature before addition to the plate. **Do not pre-mix the donor and acceptor beads.**

Streptavidin coated Donor beads

In 490 µL of Assay Buffer, add 10 µL of the AlphaScreen Donor beads. Mix gently. **Do not pre-mix the donor and acceptor beads.**

Method:

To a 384-well white opaque Optiplate™-384* :
(PerkinElmer part number 6007290)

- Add 10 µL of Acceptor Beads Mix (anti-cGMP antibody 1: 5,000 final dilution, Protein A coated Acceptor beads 20 µg/mL final conc. in 25 µL).**
- Add 5 µL cGMP competitor dilutions (0.1 nM - 1 µM final conc. in 25 µL);** Include a buffer only control.
- Add 5 µL biotinylated cGMP (1 nM final conc. in 25 µL).** Wait 5 minutes.
- Add 5 µL Donor beads (streptavidin coated Donor beads 20 µg/mL final conc. in 25 µL).**

Incubate** in the dark*** at room temperature for at least 60 minutes and analyze on either the Envision™, Fusion™-α or AlphaQuest®-HTS microplate analyzer.

Recommendation

For certain assays, it may be advantageous to increase the incubation time after addition of the beads to obtain higher total counts. In such cases, it is recommended to conduct a time course experiment to determine the optimal incubation period of the assay.

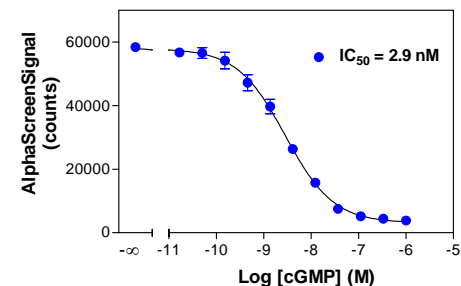
* It is recommended that the following steps be performed in triplicate.

** Plates can be covered with TopSeal™ (PerkinElmer part number 6005250) adhesive sealing film to reduce evaporation during incubation.

*** Plates can be covered by another microplate.

Result:

AlphaScreen cGMP Standard curve



The above graph illustrates a typical AlphaScreen cGMP standard curve performed as described (25 µL final volume in a 384-well microplate) at room temperature (22-23°C), 1 hour incubation then read on an EnVision. Total signal will vary with ambient temperature and incubation time. For consistent results, the same incubation time and temperature should be used for each plate.

Final concentrations of reagents in the assay are 20 µg/mL Donor beads, 20 µg/mL Acceptor beads, 1 nM biotinylated cGMP, 1:5,000 diluted anti-cGMP antibody and 0.1 nM - 1 µM cGMP.

Precautions

- The AlphaScreen cGMP Supplement should be used at the recommended final concentration of 1 nM. Higher concentrations of probe could also be used to increase total signal output. However, altering probe concentrations from the recommended 1 nM can lead to a shift in the IC₅₀ value.
- Do not use the control buffer from the AlphaScreen Protein A kit, as this will result in a decrease in total counts. Use the recommended HEPES-based buffer instead.
- Short incubation times (e.g. 1 hour at ambient temperature) are sufficient to produce robust AlphaScreen signals. Increasing the incubation time to an overnight period allows for ensuring equilibrium is reached and maximal signal is obtained. However, the higher signal generated is associated with a shift in IC₅₀ value towards a moderate decrease in sensitivity.
- AlphaScreen beads are light sensitive.
 - Take care to not expose beads to bright light. Beads are best handled under subdued or green filtered light conditions.
 - Incubation steps involving the beads should be performed in the dark. Plates can be covered by another microplate to minimize the effect of light.