cGMP SPA Biotrak™ screening assay system (dual range)

code RPA 557
5x96 wells

STORAGE
Store at 2-8°C

EXPIRY
The expiry date is stated on the package and will be at least 4 weeks from the date of despatch.

Warning: For research use only.
Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Amersham Biosciences

COMPONENTS OF THE ASSAY SYSTEM

The pack contains the following assay components, sufficient material for 5x96 wells. All components for this kit should be stored at 2-8°C.

Assay buffer
Assay buffer concentrate. On dilution the contents of this bottle will produce a 0.05M acetate buffer containing 0.01% sodium azide (see safety data sheet on pp.38-39).

Standard (for non-acetylation assay), 2 vials
cGMP standard 1.28nmol, for assays in the range 0.2-25.6pmol/well, lyophilised. On reconstitution each bottle contains 512pmol/ml.

Standard (for acetylation assay), 3 vials
cGMP standard, 25.6pmol, for assays in the range 4-512fmol/well, lyophilised. On reconstitution each bottle contains 10.24pmol/ml.

Tracer, 2 vials
Guanosine 3',5'-cyclic phosphoric acid 2'-0-succinyl-3-[^{125}I]iodotyrosine methyl ester ~103kBq, 2.8µCi, lyophilised.

Antiserum
Rabbit anti-succinyl cGMP serum, lyophilised.

Acetic anhydride, 2 vials
3ml per bottle, ready for use (see safety data sheet on pp.40-41).

Triethylamine, 2 vials
5ml per bottle, ready for use (see safety data sheet on pp.42-43).

SPA anti-rabbit reagent
Donkey anti-rabbit IgG coupled to SPA fluomicrospheres, lyophilised.

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SAFETY WARNINGS AND PRECAUTIONS

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Caution: Radioactive material

Instructions relating to the handling, use, storage and disposal of radioactive materials.

1) Upon receipt, vials or ampoules containing radioactive material should be checked for contamination. All radioactive materials should be stored in specially designated areas and suitable shielding should be used where appropriate. Access to these areas should be restricted to authorised personnel only.

2) Radioactive material should be used by responsible persons only in authorised areas. Care should be taken to prevent ingestion or contact with skin or clothing. Protective clothing, such as laboratory overalls, safety glasses and gloves should be worn whenever radioactive materials are handled. Where this is appropriate, the operator should wear personal dosimeters to measure radiation dose to the body and fingers.

3) No smoking, drinking or eating should be allowed in areas where radioactive materials are used. Avoid actions that could lead to the ingestion of radioactive materials, such as the pipetting of radioactive solutions by mouth.

4) Vials containing radioactive materials should not be touched by hand; wear suitable protective gloves as normal practice. Use forceps when handling vials containing 'hard' beta emitters such as phosphorus-32 or gamma emitting labelled compounds. Ampoules likely to contain volatile radioactive compounds should be opened only in a well ventilated fume cabinet.

5) Work should be carried out on a surface covered with absorbent material or in enamel trays of sufficient capacity to contain any spillage. Working areas should be monitored regularly.

6) Any spills of radioactive material should be cleaned immediately and all contaminated materials should be decontaminated or disposed of as radioactive waste via an authorised route. Contaminated surfaces should be washed with a suitable detergent to remove traces of radioactivity.

7) After use, all unused radioactive materials should be stored in specifically designated areas. Any radioactive product not required or any materials that have come into contact with radioactivity should be disposed of as radioactive waste via an authorised route.

8) Hands should be washed after using radioactive materials. Hands and clothing should be monitored before leaving the designated area, using appropriate instruments to ensure that no contamination has occurred. If radioactive contamination is detected, hands should be washed again and rechecked. Any contamination persisting on hands and clothing should be reported to the responsible person so that suitable remedial actions can be taken.

9) Certain national/international organisations and agencies consider it appropriate to have additional controls during pregnancy. Users should check local regulations.

Most countries have legislation governing the handling, use, storage, disposal and transportation of radioactive materials. The instructions set out above complement local regulations or codes of practice. Such regulations may require that a person be nominated to oversee radiological protection. Users of radioactive products must make themselves aware of and observe regulations or codes of practice which relate to such matters.

Warning: contains sodium azide. See safety data sheet on pp.38-39.

Warning: contains acetic anhydride. See safety data sheet on pp.40-41.

Warning: contains triethylamine. See safety data sheet on pp.42-43.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water (see safety data sheets for specific advice).

DESCRIPTION

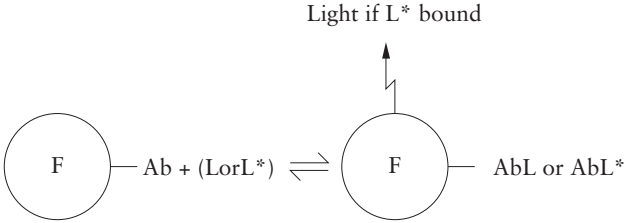
The Biotrak™ cGMP SPA screening assay system from Amersham Biosciences has been specifically designed for research purposes. This assay system utilises the novel technique of scintillation proximity assay (SPA), which eliminates the need to separate antibody bound from free ligand common to heterogeneous radioimmunoassays. The SPA fluomicrospheres used in this assay are second generation polyvinyl toluene (PVT) based beads. These remain in suspension for relatively longer periods of time when compared to the first generation yttrium silicate based SPA beads. This greatly improves pipetting accuracy and facilitates complete automation of the assay.

cGMP may be measured in the range 0.2-25.6pmol/well (1.38-176.7ng/ml), but higher sensitivity may be obtained by acetylation of standards and unknowns prior to assay. Using this approach, standard curves ranging from 4-512fmol/well (0.028-3.5ng/ml) are obtained.

Alternative protocols for assay automation and increased sensitivity are provided on pp.26-27.

Each pack contains sufficient material for 5x96 wells.
A separate standard curve should be constructed for each plate, allowing 38 unknowns to be measured in duplicate per plate. This would allow testing of 190 unknowns in duplicate.

- Choice of assay ranges - an acetylation protocol offers high sensitivity
- Automatable
- Higher sensitivity (2-8°C) protocols included
- No drift with room temperature assays
- Two additions with automation protocol (see p.26)
- Sample collection and preparation procedures included
- No separation or aspiration steps



F= PVT fluomicrosphere with anti-rabbit second antibody
Ab= primary rabbit cGMP antibody
L*= labelled ligand - [^{125}I]cGMP
L= unlabelled ligand - sample cGMP

Figure 1. Assay principle

CRITICAL PARAMETERS

The following points are critical.

- Do not agitate the plates during the course of the assay incubation period.
- A separate standard curve must be run on each plate.
- The scintillation proximity assay fluomicrospheres are insoluble. The bottle contents should be magnetically stirred to ensure a homogeneous suspension when pipetting into the wells. This is particularly important during any automation process. The fluomicrospheres must not be allowed to settle.
- When researchers are using highly coloured samples, colour quench correction may be necessary. Please contact your local Amersham Biosciences representative for further information.
- Removal of organic solvents from samples must be carried out prior to assay.

ADDITIONAL EQUIPMENT AND REAGENTS REQUIRED

- Microplate liquid scintillation counter
- 96 well microtitre plates compatible with the scintillation counter
- Plate sealers
- Disposable polypropylene tubes (12x75mm) for preparation of working standards in the non-acetylation assay
- Disposable polypropylene or glass tubes (12x75mm) for recommended acetylation procedure
- Pipettes or pipetting equipment (25µl, 50µl, 500µl, 1ml, 2.5ml, 5ml)
- Glass measuring cylinder (2000ml)
- Distilled or deionised water
- Test tube rack to hold tubes
- Vortex mixer
- Magnetic stirrer

Disposable polypropylene tubes are supplied by Sarstedt International Rommelsdore, 5223 Numbrecht, Germany.
Disposable Borosilicate glass tubes are available from Kimble, Division of Owen Illinois, Toledo, Ohio 43666 USA.
Microplate liquid scintillation counters are available from Packard Instrument Co. Inc., Meriden, CT 06450 and Wallac Oy, Finland.
Compatible 96 well microtitre plates and plate sealers are available from a wide range of suppliers such as Dynatech Laboratories, Inc., Chantilly, Virginia 22021, Packard and Wallac.

This assay system is designed for use in a microtitre plate format. However, assays can be carried out in mini scintillation vials. These are available from a wide range of suppliers such as Pharmacia-LKB, Packard and Beckman Instruments, for example Bio-Vial™ (13x55mm). These vials are counted in

traditional rack liquid scintillation counters. Do not add scintillant to the vials.

Plates used with the TopCount™ and MicroBeta™ liquid scintillation counter need to be sealed with an adhesive clear plastic. These should be ordered with the plates along with a roller to seal the plates effectively.

SPECIMEN COLLECTION AND SAMPLE PREPARATION

Numerous procedures have been described for the extraction of cGMP from biological samples. These include acidic extraction procedures using trichloroacetic acid, perchloric acid, dilute hydrochloric acid and extraction with aqueous ethanol. Some investigators also recommend the use of ion exchange chromatography following one of these extraction techniques.

Representative procedures are described below for the extraction of cGMP from tissues and cell cultures. However, it remains the responsibility of the investigator to validate the chosen extraction procedure.

Tissue samples

Tissue sections must be rapidly frozen immediately after collection so as to prevent alterations to cGMP and associated enzymes before analysis. This is usually achieved by immersion of the fresh tissue in liquid nitrogen at -196ºC.

Samples should be stored at -15ºC to -30ºC until the assay is conducted.

Extraction procedure

1) Homogenise frozen tissue in cold 6%(w/v) trichloroacetic acid at 2-8ºC to give a 10%(w/v) homogenate.

2) Centrifuge at 2000xg for 15 minutes at 4ºC.

3) Recover the supernatant and discard the pellet.

4) Wash the supernatant 4 times with 5 volumes of water saturated diethyl ether. The upper ether layer should be discarded after each wash.

5) The aqueous extract remaining should be lyophilised or dried under a stream of nitrogen at 60ºC.

6) Dissolve the dried extract in a suitable volume of assay buffer prior to analysis.

Cell suspension

Extraction procedure

1) Add ice-cold ethanol to cell suspension to give a final suspension volume of 65%(v/v) ethanol. Allow to settle.

2) Draw off the supernatant into a test tube.

3) Wash the remaining precipitate with ice cold 65%(v/v) ethanol and add the washings to the appropriate tube.

4) Centrifuge the extracts at 2000xg for 15 minutes at 4ºC and transfer the supernatant to a fresh tube.

5) Dry the combined extracts under a stream of nitrogen at 60ºC or in a vacuum oven.

6) Dissolve the dried extracts in a suitable volume of assay buffer prior to analysis.

ASSAY PROTOCOL

Reagent preparation

Storage

Before reconstitution store the reagents at 2-8°C. The expiry date is stated on the package and will normally be at least four weeks from the date of despatch.

After reconstitution the scintillation proximity assay reagent will be stable for up to 4 weeks stored at 2-8°C and protected from light. All other reconstituted components should be stored at 2-8°C and may be re-used within 14 days of reconstitution.

Note: All reagents should be allowed to equilibrate to room temperature. Either distilled or deionised water may be used for reagent preparation. Acetic anhydride and triethylamine are supplied ready for use.

Assay buffer

1. Transfer the contents of the bottle to a 2000ml graduated cylinder by repeated washing with distilled water.
2. Adjust the final volume to 1250ml with distilled water and mix thoroughly. The diluted buffer contains 0.05M acetate buffer pH5.8 containing 0.01%(w/v) sodium azide.

Standard (for non-acetylation assay)

1. Add 2.5ml diluted assay buffer to the vial and replace the stopper.
2. Gently mix until the contents are completely dissolved. The final solution contains cGMP at a concentration of 512pmol/ml.

Standard (for acetylation assay)

1. Add 2.5ml diluted assay buffer to the vial and replace the stopper.
2. Gently mix until the contents are completely dissolved. The final solution contains cGMP at a concentration of 10.24pmol/ml.

Tracer

1. Add 14ml diluted assay buffer to the vial and replace the stopper.
2. Gently mix until the contents are completely dissolved. The final solution contains guanosine 3',5'-cyclic phosphoric acid 2'-0-succinyl-3-[^{125}I]iodotyrosine methyl ester.

Antiserum

1. Add 27.5ml diluted assay buffer to the vial and replace the stopper.
2. Gently mix until the contents are completely dissolved. The final solution contains anti cGMP serum.

Note: If non-acetylation curves are prepared, an aliquot of the antiserum solution should be diluted with an equal volume of assay buffer prior to use.

SPA anti-rabbit reagent

1. Add 30ml of diluted assay buffer to the bottle and replace the stopper.
2. Gently shake the bottle for 5 minutes.

Non-acetylation assay
(range 0.2-25.6pmol/well).

Preparation of working standards

Note: It is important to use a clean pipette tip for each dilution.

1) Label 7 polypropylene tubes (12x75mm), 0.2pmol, 0.4pmol, 0.8pmol, 1.6pmol, 3.2pmol, 6.4pmol and 12.8pmol.

2) Pipette 500µl of assay buffer into all the tubes.

3) Into the 12.8pmol tube pipette 500µl of stock standard (512pmol/ml) and mix thoroughly.

4) Transfer 500µl from the 12.8pmol tube to the 6.4pmol tube and mix thoroughly.

5) Repeat this doubling dilution successively with the remaining tubes.

6) 50µl aliquots from each serial dilution will give rise to 7 standard levels of cGMP ranging from 0.2-12.8pmol/well.

7) 50µl from the stock standard vial will give rise to the 25.6pmol standard.

Assay procedure

1) Prepare assay buffer and standards ranging from 0.2-25.6pmol/50µl as described in the previous section.

2) Equilibrate all reagents to room temperature and mix before use.

3) Set up the plate to enable the running in duplicate of all non-specific binding (NSB), zero standard (B_o), standards and samples.

4) Pipette 100µl of assay buffer into the non-specific binding wells (NSB).

5) Pipette 50µl of assay buffer into the zero standard wells (B_o).

6) Starting with the most dilute, pipette 50µl of each standard into the appropriate wells, using a clean pipette tip for each standard.

7) Pipette 50µl of each unknown sample into appropriate wells. See sample preparation section.

8) Pipette 50µl of [^{125}I]cGMP into all wells.

9) Pipette 50µl of antiserum into all wells except the non-specific binding (NSB).

Note: Antiserum for the preparation of non-acetylation curves should be diluted with an equal volume of assay buffer prior to use.

10) Place the SPA anti-rabbit reagent on to a magnetic stirrer and adjust the stirring speed to ensure a homogeneous suspension. Then add 50µl to all wells.

11) All wells should now contain a total volume of 200µl.

12) Seal the plates and incubate at room temperature (15-30°C) for 15-20 hours.

13) Determine the amount of [^{125}I]cGMP bound to the fluomicrospheres by counting in the microtitre plate β scintillation counter for 2 minutes.

Acetylation assay
(range 4-512fmol/well)

Preparation of working standards

Note: It is important to use a clean pipette tip for each dilution.

1) Label 8 polypropylene or glass tubes (12x75mm) 4fmol, 8fmol, 16fmol, 32fmol, 64fmol, 128fmol, 256fmol and 512fmol.

2) Pipette 500µl of assay buffer into all the tubes except the 512fmol tube.

3) Pipette 500µl of stock acetylation standard (10.24pmol/ml) into the 512fmol tube and 500µl into the 256fmol tube and mix thoroughly.

4) Transfer 500µl from the 256fmol tube into the 128fmol tube and vortex mix thoroughly.

5) Repeat this doubling dilution successively with the remaining tubes.

6) Remove 500µl from the 4fmol standard and discard. All tubes should now contain 500µl.

7) 50µl aliquots from each serial dilution will give rise to 8 standard levels of cGMP ranging from 4 to 512fmol/well.

Note: These tubes will be referred to as acetylation tubes containing working standards in the assay protocol section. Working standards should be freshly prepared before each assay and not re-used.

Assay procedure

Note: Steps 7-15 should be performed as quickly as possible. Only polypropylene or glass tubes should be used for steps 1-8.

1) Prepare assay buffer and standards ranging from 4-512fmol/well as described in the previous section.

2) Equilibrate all reagents to room temperature and mix before use.

3) Label polypropylene or glass tubes (12x75mm) for non-specific binding, zero standard and unknowns. These will subsequently be known as acetylation tubes.

4) Set up the plate to enable the running in duplicate of all non-specific binding (NSB), zero standard (B_o), standards and samples.

5) Prepare the acetylation reagent by mixing 1 volume of acetic anhydride with 2 volumes of triethylamine in a glass vessel. Mix well. (Sufficient reagent for 50 acetylations may be attained by mixing 0.5ml acetic anhydride with 1.0ml triethylamine).

6) Pipette 500µl assay buffer into each of the non-specific binding and zero standard acetylation tubes.

7) Pipette 500µl of each unknown (see sample preparation section) into appropriately labelled acetylation tubes. Tubes containing 500µl of each working standard should already have been prepared (see reagent preparation section).

8) Carefully add 25µl of the acetylation reagent to all acetylation tubes containing non specific binding, zero standard, standards and unknowns. Optimum precision is attained by placing the pipette tip in contact with the test tube wall above the aqueous layer and allowing the acetylation reagent to run down the test tube wall into the liquid. Each tube should be vortexed immediately following addition of the acetylating reagent.

9) Pipette duplicate 50µl aliquots from all acetylation tubes into the appropriate wells.

10) Pipette 50µl of [^{125}I]cGMP tracer into all wells.

11) Pipette 50µl of antiserum into all wells except the NSB.

12) Pipette 50µl of assay buffer into the NSB wells.

13) Place the SPA anti-rabbit reagent on to a magnetic stirrer and adjust the stirring speed to ensure a homogeneous suspension. Then add 50µl to all wells.

14) All wells should now contain a total volume of 200µl.

15) Seal the plates and incubate at room temperature (15-30°C) for 15-20 hours.

16) Determine the amount of [^{125}I]cGMP bound to the fluomicrospheres by counting in the microtitre plate β scintillation counter for 2 minutes.

Counting

- Scintillant should not be added to the wells.
- Instrument settings for microtitre plate scintillation counters are described below:

a) Window settings for Microbeta *5-530*

b) **Table 1** Instrument settings for TopCount

				Window settings	
Scintillator	**Energy range**	**Efficiency mode**	**Isotope**	**Region A**	**Region B**
Liquid	Low	High	Iodine-125	0-100	0-256

- Where assays are carried out in vials, and counted on a Rack Beta counter, for those fitted with spectrum analysis packages, the suitable window opening may be determined and set accordingly. Windows for other counters should be set wide open. For example:

a) Beckman LS7800 where H# is set at 0 and the window open from 10-999.
b) Packard TriCarb 460 with the window open from 0 to 999.
c) LKB 1209/1215 Rack Beta with the window open from 5 to 999. SQP(I) and SQP(E) are not used.

DATA PROCESSING

Calculation of standard curve data

The assay data collected should be similar to the data shown in tables 2 and 3.

1) Calculate the average counts per minute (cpm) for each set of replicate wells.

2) Calculate the percent bound for each standard and sample using the following relationship:

$$\%B/B_0 = \frac{\text{(Standard or sample cpm-NSB cpm)}}{B_0 \text{ cpm - NSB cpm}} \times 100$$

A standard curve may be generated by plotting the percent B/B_o as a function of the log cGMP concentration.

3) Plot % B/B_o (y-axis) against the amount of standard per well (x-axis). The curve shape should be similar to figures 2 and 3, if plotted on semi-log paper.

The amount of cGMP per well of the unknown samples can be read directly from the graph.

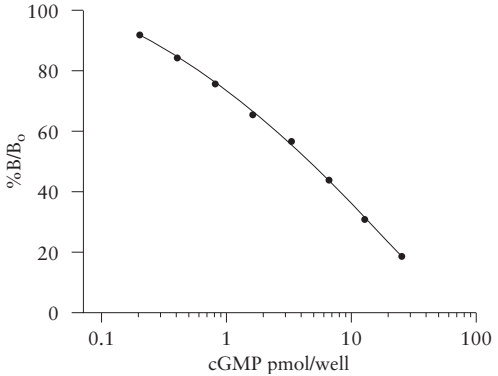


Figure 2. Typical standard curve: non-acetylation protocol.

Table 2. Typical assay data: non-acetylation protocol

Well	cpm*	Average cpm	% B/B_0**
Non-specific binding (NSB)	183 173	178	-
Zero standard (B_0)	2203 2037	2120	-
0.2pmol standard	1985 1952	1968	92.2
0.4pmol standard	1797 1833	1815	84.3
0.8pmol standard	1709 1599	1654	76.0
1.6pmol standard	1488 1420	1454	65.7
3.2pmol standard	1234 1324	1279	56.7
6.4pmol standard	1058 1004	1031	43.9
12.8pmol standard	789 761	775	30.7
25.6pmol standard	489 587	538	18.5

These data were calculated using Dynatech Microlite-1 plates counted on a TopCount microplate scintillation counter. Efficiencies of other multihead beta counters vary from this, and may give different results.

* Corrected for instrument blank

** For the calculation of % B/B_o values, non-specific binding has been subtracted from the counts.

In order to express results from unknown samples in terms of pmol/ml multiply values obtained from the standard curve by 20.

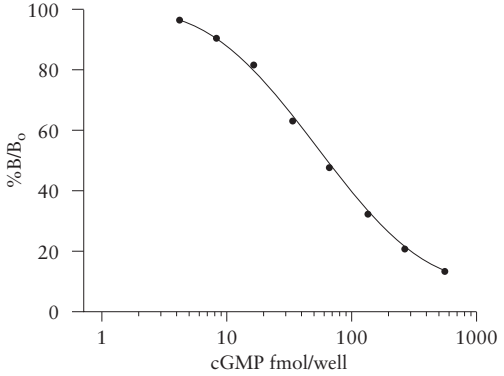


Figure 3. Typical standard curve: acetylation protocol.

Table 3. Typical assay data: acetylation protocol

Well	cpm*	Average cpm	% B/B_0**
Non-specific binding (NSB)	170 171	171	-
Zero standard (B_0)	2273 2277	2275	-
4fmol standard	2174 2251	2212	97.0
8fmol standard	2109 2052	2080	90.7
16fmol standard	2004 1790	1897	82.0
32fmol standard	1568 1451	1510	63.6
64fmol standard	1185 1198	1192	48.5
128fmol standard	841 906	873	33.4
256fmol standard	627 622	625	21.6
512fmol standard	463 481	472	14.3

These data were calculated using Dynatech Microlite-1 plates counted on a TopCount microplate scintillation counter. Efficiencies of other multihead beta counters vary from this, and may give different results.

* Corrected for instrument blank

** For the calculation of % B/B_o values, non-specific binding has been subtracted from the counts.

In order to express results from unknown samples in terms of pmol/ml multiply values obtained from the standard curve by 20.

ADDITIONAL APPLICATIONS

Alternative protocol for assay automation

Preparation of reagents

Prepare assay reagents and working standards as described previously. Add equal volumes of tracer, antiserum and SPA anti-rabbit reagent to a beaker, ensuring that sufficient reagent is prepared to run the desired number of wells (eg 1ml tracer, 1ml antiserum and 1ml SPA anti-rabbit reagent should provide sufficient mixture for 20 wells). This working solution should be freshly prepared before each assay and not be re-used.

Assay procedure

Note: It is recommended that non-specific binding wells (NSB) are excluded from this protocol because of the addition of antiserum to the working solution. Further dilution of the antiserum is also required if the non-acetylation assay protocol is used and this must be performed prior to pooling the assay reagents for the working solution. If the acetylation assay protocol is used, standards and samples must be acetylated in the usual way as described on p.19 of the pack leaflet.

1) Pipette 50µl of assay buffer into the zero standard wells (B_o).

2) Starting with the most dilute, pipette 50µl of each standard into the appropriate wells.

3) Pipette 50µl of each unknown sample into appropriate wells. See sample preparation section.

4) Place the working solution (containing equal volumes of tracer, antiserum and SPA anti rabbit reagent) on to a magnetic stirrer and adjust the stirring speed to ensure a homogeneous suspension. Add 150µl to all wells, which should now contain a total volume of 200µl.

5) Seal, incubate and count the plates as described previously in the pack leaflet.

6) Calculate results as described previously.

Note: This protocol is not routinely quality controlled and the data not presented in the pack leaflet.

Alternative protocol for increased sensitivity

Preparation of reagents

Assay reagents and working standards should be prepared as described previously in the pack leaflet (refer to assay protocol section).

Assay procedure

Perform the assay as described in the assay protocol section of the pack leaflet. However the plates should be incubated at 2-8°C for 15-20 hours instead of room temperature.

Important

It is imperative that the microtitre plates are counted immediately or within a maximum of 3 hours following completion of the incubation period. Otherwise the assay will re-equilibrate to ambient laboratory temperature resulting in a loss of sensitivity and possible assay drift.

Note: This protocol is not routinely quality controlled and the data not presented in the pack leaflet.

ADDITIONAL INFORMATION

Specificity

The cross-reactivity, as determined by the concentration giving 50% B/B_o with a number of related compounds is shown in table 4.

Table 4.

Compound	Cross-reactivity %	
	Non-acetylation	Acetylation
cGMP	100	100
cAMP	0.0049	0.0074
AMP	<0.00044	<0.0000054
ADP	<0.00044	<0.0000054
ATP	<0.00044	<0.0000054
GMP	0.2	0.00096
GDP	0.09	0.00048
GTP	0.04	0.00016

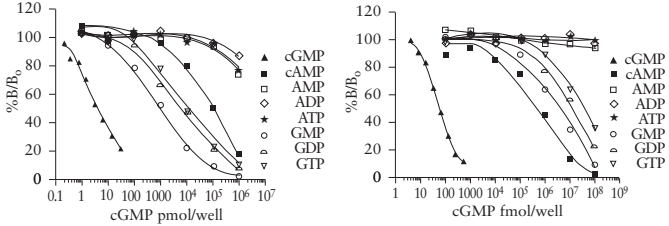


Figure 4. (a) Cross-reactivity profile for the non-acetylation assay

(b) Cross-reactivity profile for the acetylation assay

Sensitivity

The sensitivity, defined as the amount of cGMP needed to reduce the zero dose binding by two standard deviations, was as follows:

Acetylation protocol	4fmol/well	27.6pg/ml
Non-acetylation protocol	0.2pmol/well	1.38ng/ml

Precision

The within-assay precision for duplicate determinations was calculated by measuring controls in the assay. The results are shown below.

Table 5. Non-acetylation (mean values as pmol/well)

Control	Mean ± SD	% CV	n
J	1.00±0.14	13.7	20
K	2.41±0.20	8.1	20
L	9.07±0.51	5.6	20

Table 6. Acetylation (mean values as fmol/well)

Control	Mean ± SD	% CV	n
P	13.8±1.28	9.3	20
Q	44.9±2.79	6.2	20
R	189.5±8.69	4.6	20

The between assay precision was assayed by repeated measurement of the same control in successive assays. The results are shown below.

Table 7. Non-acetylation protocol (mean values as pmol/well)

Control	Mean ± SD	% CV	n
J	0.93±0.12	13.3	22
K	2.87±0.35	12.3	22
L	9.57±0.85	8.9	22

Table 8. Acetylation protocol (mean values as fmol/well)

Control	Mean ± SD	% CV	n
P	13.5±2.1	15.3	22
Q	48.6±4.8	9.9	22
R	176.0±14.0	8.0	22

Precision profile

A precision profile was generated for each protocol by preparing replicates of each of the standards and calculating the standard deviation and percent coefficient of variation at each concentration.

Table 9. Non-acetylation protocol

Standard (pmol/well)	Standard deviation	% CV
0.2	0.057	30.1
0.4	0.156	35.8
0.8	0.112	14.0
1.6	0.110	7.0
3.2	0.345	10.3
6.4	0.421	7.0
12.8	1.002	7.8
25.6	1.984	7.7

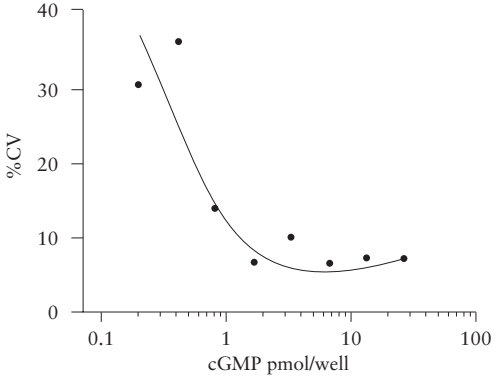


Figure 5. Precision profile - Non-acetylation protocol

Table 10. Acetylation protocol

Standard (fmol/well)	Standard deviation	% CV
4	1.435	39.2
8	1.659	18.4
16	2.361	16.1
32	2.341	8.0
64	2.842	4.3
128	5.361	4.2
256	24.29	9.4
512	61.5	12.8

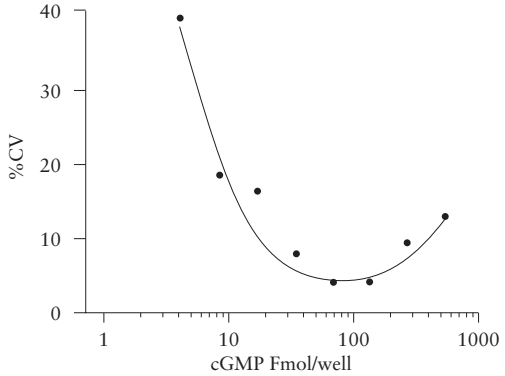


Figure 6. Precision profile - Acetylation protocol

Troubleshooting guide

Problem	Check
Poor replication	• Reduce the pipetting speed. Use 'contain' pipetting with tip touch at source and destination. • Use a pre-wet cycle. This improves replicates when using small volumes (eg. 50µl). • Ensure that a homogeneous suspension of fluomicrospheres is maintained. • If replication is still poor, calibrate tools. If no improvement is gained, contact supplier of equipment.

Background and references

cGMP was first identified by Ashman *et al,* in 1963[1]. Since then it has been shown to be widely distributed, occurring at low levels (1-10% of cAMP levels) in most animal tissues.

A number of roles have been suggested for cGMP based on changes in cGMP levels in tissues after challenging with various ligands[2,3]. For example acetylcholine, oxytocin, insulin, serotonin and histamine cause an increase in intracellular cGMP levels. Vasodilators such as nitroprusside, nitroglycerine, sodium nitrate and nitric oxide (NO), putatively identified as Endothelium-derived relaxing factor (EDRF), also increase cGMP levels via stimulation of soluble guanylate cyclase[4]. Consequently cGMP has been implicated as an effector of smooth muscle relaxation. This has stimulated interest in selective cGMP phosphodiesterase inhibitors as potential antihypertensives[5,6].

It is well established that cGMP is involved in the retinal photo-transduction system[7]. An entirely new type of ion channel structure has been proposed in the recently cloned cGMP-gated channel of the rod photoreceptor[8].

Another important role for cGMP is in the action of atrial natriuretic peptide (ANP). Atrial peptides have the ability to relax smooth muscle which is accompanied by increased intracellular cGMP levels. Recent work is confirming cGMP as a second messenger for ANP[9]. Cloning of rat brain membrane guanylate cyclase cDNA has shown it to have both ANP receptor and guanylate cyclase domains[10]. An area of great interest at present centres on NO. An increasing number of mammalian cells including vascular endothelium can synthesise NO from L-arginine[11,12]. NO has the ability to diffuse through cell membranes. This means the NO-haem-guanylate cyclase system represents an unique signal transduction mechanism linking extracellular stimuli to the synthesis of cGMP in nearby target cells. In this system it is suggested that cGMP acts as a third messenger[12].

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Related products

Casein kinase II enzyme assay	RPN 86
cdc2 kinase enzyme assay	RPN 83
cAMP enzyme immunoassay (EIA) system (dual range)	RPN 225
cAMP [^{3}H]assay system	TRK 432
cAMP [^{125}I] assay system (dual range)	RPA 509
cAMP [^{125}I] scintillation proximity assay (SPA) system (dual range) 100 tubes	RPA 538
cAMP [^{125}I] scintillation proximity assay (SPA) system (dual range) 500 tubes	RPA 542
cAMP [^{125}I] SPA screening assay system (5x96 wells)	RPA 556
cGMP enzyme immunoassay (EIA) system (dual range)	RPN 226
cGMP [^{3}H]assay system	TRK 500
cGMP [^{125}I] assay system (dual range)	RPA 525
cGMP [^{125}I] scintillation proximity assay (SPA) system (dual range) 100 tubes	RPA 540
cGMP [^{125}I] scintillation proximity assay (SPA) system (dual range) 500 tubes	RPA 541
sn-1,2-Diacylglygerol (DAG) assay system	RPA 200
ds DNA-activated protein kinase enzyme assay	RPN 85
EGF receptor tyrosine kinase enzyme assay	RPN 78
D-*myo*-Inositol 1,4,5-trisphosphate (IP_3) [^{3}H] assay system	TRK 1000
MAP kinase enzyme assay	RPN 84

Protein kinase C enzyme assay	RPN 77
Amprep™ SAX 500mg (pack of 50)	RPN 1918
Amprep SAX 100mg (pack of 100)	RPN 1908
Amprep column adaptor (pack of 15)	RPN 1927
Amprep reservoir (pack of 100)	RPN 1929
Amprep manifold-10	RPN 1930
Amprep super-separator-24	RPN 1940

Scintillation proximity assay reagents

Anti-rabbit	RPN 140
Anti-mouse	RPN 141
Anti-sheep	RPN 142
Protein A	RPN 143

Safety data sheets contacts

Australia
Sydney
(02)894-5188

Belgium
Gent
(092)41-52-70

Canada
Oakville
(905)847-1166

Denmark
Birkerød
82-02-22

Asia Pacific
Hong Kong
2802-1288

France
Les Ulis
(1)69-1828-00

Germany
Braunschweig
(05307)930-0

Iberica
Madrid
(1)304-42-00

Italy
Milan
(02)5088-220

Japan
Tokyo
(03)38-16-1091

Nederland
s'Hertogenbosch
(073)641-85-25

Norway
Gjettum
(67)54-63-18

Sweden
Solna
(08)444 7180

UK Sales
Little Chalfont
(0800)515313

USA
Arlington Heights
IL (708)593 6300

Safety data sheet

SDS201/AB Date of issue Feb 1999

Amersham Biosciences UK Limited Amersham Place Little Chalfont
Buckinghamshire England HP7 9NA Telephone: +44 (0)870 606 1921

Product name:	Sodium azide	CAS No:26628-22-8
	R: 22-32	Harmful if swallowed. Contact with acids liberates very toxic gas.
	S: (1/2)-28-45	(Keep locked up and out of the reach of children). After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).
Composition:	Aqueous sodium azide solution (0.1-0.99%).	
Hazards identification:	Harmful if swallowed, inhaled or absorbed through skin. May cause eye and skin irritation.	
First aid measures:	In case of contact, immediately flush eyes or skin with copious amounts of water. If inhaled remove to fresh air. In severe cases seek medical attention.	
Fire fighting measures:	Dry chemical powder. Do not use water.	
Accidental release:	Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Mop up spill area, place waste in a bag and hold for waste disposal. Wash spill site area after material pick up is complete.	

Handling and storage:	Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Do not get in eyes, on skin or on clothing. Wash thoroughly after handling.
Personal protection:	See above instructions for handling and storage.
Physical and chemical properties:	Formula weight: 65.01. Density: 1.850.
Stability and reactivity:	Avoid contact with metals and acid chlorides. This yields a very toxic gas.
Toxicological information:	LD_{50}: 27mg/kg oral, rat. LD_{50}: 20mg/kg skin, rabbit.
Ecological information:	Not applicable.
Disposal considerations:	Up to 5 vials worth of material may be disposed of directly down the sink with water. If 6 or more vials are to be disposed of they should pass through a chemical waste route.
Transport information:	No special considerations applicable.
Regulatory information:	The information contained in this safety data sheet is based on published sources and is believed to be correct. It should be used as a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.

For further information contact your local office. See page 37.

Safety data sheet SDS207/AB Date of issue Feb 1999

Amersham Biosciences UK Limited Amersham Place, Little Chalfont
Buckinghamshire England HP7 9NA Telephone: +44 (0)870 606 1921

Product name: Acetic anhydride CAS No: 108-24-7

R: 10-34 Flammable. Causes burns.

S: (1/2)26-45 (Keep locked up and out of the reach of children). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Composition: Acetic anhydride concentrate.

Hazards identification: Flammable. Causes burns. Harmful if swallowed, inhaled or absorbed through the skin. Material is destructive to the tissues of the mucous membranes, upper respiratory tract, eyes and skin.

First aid measures: In case of contact, immediately flush eyes or skin with copious amounts of water. Remove articles of contaminated clothing and shoes. Ensure adequate flushing of contaminated eyes by separating the eyelids with fingers. If inhaled remove to fresh air. In severe cases seek medical attention.

Fire fighting measures: Carbon dioxide, dry chemical powder or polymer foam.

Accidental release: Wear suitable laboratory protective equipment; lab coats, gloves and safety glasses. Cover with activated carbon absorbent. Take up and place in a closed container. Transport outdoors. Ventilate area and wash spill after material pick-up is complete.

Handling and storage:	Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Do not get in eyes, on skin or on clothing. Do not breathe the vapour. Avoid prolonged repeated exposure. Wash thoroughly after handling. Keep container away from heat and naked flame. Store in a cool, dry place. Combustible.
Personal protection:	See above instructions for handling and storage.
Physical and chemical properties:	Melting point -73°C. Boiling point: 138-140°C. Vapour pressure: 4.6mm (20°C). Lower explosion limit: 2.8. Upper explosion limit: 10.3. Flash point: 130°F(54°C). Formula weight: 102.09. Density: 1.080. Vapour density: 3.52. Auto-ignition temperature: 629°F.
Stability and reactivity:	Acids, bases, moisture, alcohols, oxidising agents, reducing agents and finely powdered metals.
Toxicological information:	Skin-rabbit: 10mg/24h open mld. Skin-rabbit: 540mg open mld. Eye-rabbit: 250µg open sev.
Ecological information:	Not applicable.
Disposal considerations:	Up to 5 vials worth of material may be disposed of directly down the sink with water. If 6 or more vials are to be disposed of they should pass through a chemical waste route.
Transport information:	No special considerations applicable.
Regulatory information:	The information contained in this safety data sheet is based on published sources and is believed to be correct. It should be used as a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.

For further information contact your local office. See page 37.

Safety data sheet SDS208/AB Date of issue Feb 1999

Amersham Biosciences UK Limited Amersham Place Little Chalfont
Buckinghamshire England HP7 9NA Telephone: +44 (0)870 606 1921.

Product name:	Triethylamine	CAS No: 121-44-8
	R: 11-36-37	Highly flammable. Irritating to eyes. Irritating to respiratory system.
	S: (2-) 16-26-29	(Keep out of reach of children). Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
Composition:	Colourless liquid.	
Hazards identification:	Highly flammable. Irritating to the eyes. Irritating to the respiratory system. Harmful if swallowed, inhaled or absorbed through the skin. Causes burns.	
First aid measures:	In case of contact, immediately flush eyes or skin with copious amounts of water. Remove contaminated clothing and shoes. If inhaled remove to fresh air. In severe cases seek medical attention.	
Fire fighting measures:	Carbon dioxide, dry chemical powder or polymer foam.	
Accidental release:	Shut off all sources of ignition. Wear suitable laboratory protective equipment; lab coats, gloves and safety glasses. Cover with dry lime, sand or soda ash. Place in covered containers using non-sparking tools and transport outdoors.	
Handling and storage:	Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Do not get in eyes, on skin or on clothing. Do not breathe the vapour. Wash thoroughly after handling.	

	Keep tightly closed. Keep away from heat, sparks and open flame.
Personal protection:	See above instructions for handling and storage.
Physical and chemical properties:	Melting point -7°C. Boiling point: 88.8°C. Vapour pressure: 54.0mm (20°C). Lower explosion limit: 1.2. Upper explosion limit: 8.0. Flash point: 20°F. Formula weight: 101.19. Density: 0.726. Vapour density: 3.5.
Stability and reactivity:	Incompatible with acids and oxidising agents. Thermal decomposition may produce carbon monoxide, carbon dioxide, and nitrogen oxides.
Toxicological information:	Skin-rabbit: 10mg/24h open mld. Skin-rabbit: 365mg open mld. Eye-rabbit: 250µg open sev. Eye-rabbit: 50 ppm/30d-I sev. LD_{50}: 460mg/kg oral, rat. LD_{50}: 570mg/kg skin, rabbit. LC_{50}: 6g/m3/2h inh, mus.
Ecological information:	Not applicable.
Disposal considerations:	Up to 5 vials worth of material may be disposed of directly down the sink with water. If 6 or more vials are to be disposed of they should pass through a chemical waste route. Do not empty into the drains.
Transport information:	No special considerations applicable.
Regulatory information:	The information contained in this safety data sheet is based on published sources and is believed to be correct. It should be used as a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.

For further information contact your local office. See page 37.

Note: Scintillation Proximity Assay (SPA) Technology is covered by US Patent No. 4568649, European Patent No. 0154734 and Japanese Patent Application No. 84/52452.

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