cGMP [^{125}I] RIA KIT

(REF: RK-525)

For Research Use Only. Not for use in diagnostic procedures.

The [^{125}I]cGMP RIA system (dual range) provides direct quantitative *in vitro* determination of cGMP in urine, plasma, tissues and other biological samples. cGMP may be measured in the range 2–128 fmol per tube (0.7–44 pg/tube) using an acetylation protocol or 50–6400 fmol per tube (17.5–2209 pg/tube) using a non-acetylation protocol. Each pack contains sufficient material for 100 assay tubes. 42 unknowns can be measured in duplicate.

Introduction

Following the recognition that cAMP acts as an important intracellular regulator in a number of tissues, the attention of researchers inevitably turned to the role of other cyclic nucleotides including cGMP. cGMP was first identified by Ashman et al in 1963 and since that time, it has been shown to be widely distributed, occurring in most animal tissues. Levels of cGMP in most tissues are very low, being 1–10% those of cAMP.

As a result, RIA for cGMP must be both sensitive and demonstrate low cross-reactivity with cAMP. Since its discovery, a number of roles have been proposed for cGMP based on observations of changes in cGMP levels in tissues after challenging with various ligands. It has been demonstrated that a number of agents including acetylcholine, oxytocin, insulin, serotonin and histamine cause elevation of intracellular cGMP. Further, vasodilators such as nitroprusside, nitroglycerine and sodium nitrite also appear to increase cGMP levels. As a result, cGMP has been implicated as an effector of smooth muscle relaxation.

A further role for cGMP has been identified in the phototransduction system of the retina. Light stimulation of vertebrate rod photoreceptors causes a decrease in the levels of cGMP in the light sensitive regions of the cell. This effect is mediated by calcium dependent phosphodiesterases.

Perhaps the most exciting recent discovery in cGMP research is the demonstration of an apparent link between intra-and extracellular cGMP levels and circulating αANP. αANP and other atrial peptides have the ability to relax smooth muscle. Stimulation of αANP receptors in tissues such as kidney endothelial cells and vascular smooth muscle cells is characterized by increased intracellular cGMP levels (by up to 50-fold). Further, plasma and urinary cGMP have been shown to be elevated by αANP.

Considerable work remains to be done in this area before cGMP can be accepted as a second messenger for αANP, however. Sodium nitroprusside mediated elevation of intracellular cGMP in tracheal smooth muscle cells is characterized by activation of a cGMP-dependent protein kinase which in turn mediates the phosphorylation of a specific subset of intracellular proteins.

Similar effects have not been demonstrated after αANP mediated stimulation of vascular smooth muscle cells. Moreover, pharmacological levels of αANP and other atrial peptides are required to give measurable increases in cGMP. This effect has yet to be demonstrated with physiological doses of αANP. However, recent research has indicated that membrane bound guanylate cyclase is closely associated with one of the atrial natriuretic peptide receptors of rat adrenocortical carcinoma cells. The identification of endothelial derived relaxing factor (EDRF) and its interaction with soluble guanylate cyclase implies yet another important role for cGMP.

Principle of method

The assay is based on the competition between unlabelled cGMP and a fixed quantity of 125I-labelled cGMP for a limited number of binding sites on a cGMP-specific antibody. With fixed amounts of antibody and radioactive ligand, the amount if radioactive ligand bound by the antibody will be inversely proportional to the concentration of added non-radioactive ligand.

The antibody bound cGMP is then reacted with the separating second antibody reagent which contains second antibody that is bound to magnetizable polymer particles. Separation of the antibody bound fraction is effected by either magnetic separation or centrifugation of the separating reagent suspension and decantation of the supernatant.

Measurement of the radioactivity in the pellet enables the amount of labelled cGMP in the bound fraction to be calculated. The concentration of unlabelled cGMP in the sample is then determined by interpolation from a standard curve.

Contents of the kit

1. 1 vial TRACER, lyophilized, reconstitution with 11 ml assay buffer, containing ~ 50 kBq, 1.35 µCi guanosine 3',5'-cyclic phosphoric acid 2'-0-succinyl 3-[^{125}I]iodotyrosine methyl ester in 0.05 M acetate buffer, pH5.8, containing 0.01% (w/v) sodium azide after reconstitution. Store at 2-8 °C.

2. 1 vial non-acetylation STANDARD, lyophilized, reconstitution with 10.0 ml assay buffer. The final solution contains cGMP at a concentration of 128 pmol/ml in 0.05 M acetate buffer, pH5.8, containing preservative. Store at 2-8 °C.

3. 1 vial acetylation STANDARD, lyophilized, reconstitution with 10.0 ml assay buffer. The final solution contains cGMP at a concentration of 2.56 pmol/ml in 0.05 M acetate buffer, pH5.8, containing preservative. Store at 2-8°C.

4. 1 vial ANTISERUM, lyophilized, reconstitution with 11 ml assay buffer. The final solution contains rabbit anti-cGMP serum in 0.05 M acetate buffer, pH5.8, containing 1% (w/v) bovine serum albumin and 0.01% (w/v) sodium azide. Store at 2-8 °C.

5. 1 vial ASSAY BUFFER concentrate (11 ml), dilution to 500 ml. The diluted buffer contains 0.05 M acetate buffer, pH5.8, with preservative. Store at 2-8 °C.

6. 1 vial SEPARATING SECOND ANTIBODY REAGENT (58 ml), ready for use, containing donkey anti-rabbit serum coated on to magnetizable polymer particles with sodium azide, colour coded, blue-green. Store at 2-8 °C.

7. 1 vial ACETIC ANHYDRIDE (1 ml), ready for use. Caution: flammable, corrosive, causes burns. Store at 2-8 °C.

8. 1 vial TRIETHYLAMINE (2 ml), ready for use. Caution: flammable, harmful vapour. Store at 2-8 °C.

Pack leaflet

Materials, tools and equipment required

Pipettes or pipetting equipment with disposable tips (25µl, 100µl, 500 µl, 10.0 ml and 11 ml); disposable polypropylene or polystyrene tubes (12 x 75 mm) for assay procedure; disposable polypropylene or glass tubes (12 x 75 mm) for acetylation procedure; refrigerator; glass measuring cylinder (500 ml); test tube rack; vortex mixer; plastic foil; separators, comprising magnetic base and assay rack - these are for use in the magnetic separation protocol; adsorbent tissue; gamma counter.

Note: For the centrifugal protocol, the following additional equipment will be required:

Decantation racks; refrigerated centrifuge capable of 2000 xg.

Specimen collection, storage

Urine samples

Random, timed or 24-hour urine collections may be analyzed. If 24-hour samples are collected, it may be necessary to include a bacteriostat (2 ml 6 M hydrochloric acid per 100 ml urine is sufficient for this purpose). Samples analyzed within 24 hours of collection may be stored at 2–8°C until assayed. If analysis is not performed within 24 hours, all samples should be stored at -15°C to -30°C.

If urine contains particulate matter this should be removed by centrifugation prior to assay.

Plasma samples

Measurements should be made in plasma not serum. Blood should be collected into tubes containing 7.5 mM EDTA. Blood should be immediately centrifuged to remove cells and the plasma stored at -15°C to -30°C prior to analysis. If blood samples cannot be rapidly processed they should be stored in ice until it is possible to centrifuge.

Tissue and cell culture

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 -196°C.

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

Extraction procedure

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 plasma, tissue and cell culture. However, it remains the responsibility of the investigator to validate the chosen extraction procedure.

<u>Plasma and tissue</u>

1. Homogenize frozen tissue in cold 6% trichloroacetic acid at 4°C to give a 10% w/v homogenate.
2. Centrifuge at 2000 g for 15 minutes at 4°C.
3. Recover the supernatant and discard the pellet.
4. Wash the supernatent 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 lyophilized or dried under a stream of nitrogen at 60°C.
6. Dissolve the dried extract in a suitable volume of assay buffer prior to analysis.

Cell suspension

1. Add ice-cold ethanol to cell suspensions to give a final suspension volume of 65% ethanol. Allow to settle.
2. Draw off the supernatant into test tubes.
3. Wash the remaining precipitate with ice cold 65% ethanol and add the washings to the appropriate tubes.
4. Centrifuge the extracts at 2000 g for 15 minutes at 4°C and transfer supernatent to fresh tubes.
5. Evaporate the combined extracts under a stream of nitrogen at 60°C or in a vacuum oven.
6. Dissolve the dried extracts in a suitable volume of assay buffer prior to analysis.

Urine

It is not necessary to extract or deproteinize urine before analysis.
Urine should be diluted with assay buffer prior to assay.

Preparation of reagents, storage

Storage: *see Contents of the kit*. At these temperatures each reagent is stable until expiry date. The actual expiry date is given on the package label and on the quality certificate. Reconstituted components should be stored at 2–8°C and may be re-used within 28 days of dilution.

Preparation: Equilibrate all reagents and samples to room temperature prior to use.

Assay buffer: Transfer the contents of the bottle to a 500 ml graduated cylinder by repeated washing with distilled water. Adjust the final volume to 500 ml with distilled water and mix thoroughly. Assay buffer is used to reconstitute all other components.

The other components (except ready for use components): Carefully add the required volume of assay buffer and replace stopper. Mix the contents of the bottles by inversion and swirling, taking care to avoid foaming.

Preparation of working standards

1. Label 7 polypropylene or glass tubes 2 fmol, 4 fmol, 8 fmol, 16 fmol, 32 fmol, 64 fmol and 128 fmol.
2. Pipette 500 µl assay buffer into all tubes.
3. Into the top standard (128 fmol) tube pipette 500 µl stock standard and mix thoroughly.
4. Transfer 500 µl from the top standard to the next tube (64 fmol) and
mix thoroughly.
5. Repeat this doubling dilution successively with the remaining tubes.
For the acetylation assay, all tubes must contain the same volume (500 µl). Therefore, remove 500 µl from the 2 fmol standard and discard.
6. 100 µl aliquots from each serial dilution give rise to 7 standard levels of cGMP ranging from 2–128 fmol.

Assay procedure

This assay system can be used to prepare standard curves in the range 2–128 fmol/tube or 50–6400 fmol/tube. Optimum sensitivity is achieved following the method outlined below. Alternatively, less sensitive acetylation and non-acetylation assays may be prepared as outlined.

Note: Steps 7–11 should be completed as quickly as possible. Only polypropylene or glass tubes should be used for acetylation tubes. Polypropylene or polystyrene tubes may be used for assay tubes. Only acetylation assay protocol is described in detail as this is the only method routinely checked by our Quality Control Department.

(For a quick guide , refer to Table 1.)

Day 1

1. Prepare assay buffer and standards ranging from 2–128 fmol/tube as described in the previous section.
2. Equilibrate all reagents to room temperature and mix before use.
3. Label polypropylene or glass tubes (12 x 75 mm) for the zero standard tube and unknowns. These will subsequently be known as acetylation tubes.
4. Label polypropylene or polystyrene tubes (12 x 75 mm) in duplicate for total counts (TC), zero standard (Bo), each standard dilution and unknowns. These will subsequently be known as assay tubes.
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 0.5 ml acetic anhyride with 1.0 ml triethylamine).
6. Pipette **500 µl** assay buffer into the zero standard acetylation tube.
7. Pipette **500 µl** of each unknown (see sample preparation section) into the appropriately labelled acetylation tubes. Tubes containing **500 µl** of each working standard should already have been prepared (see reagent preparation section).
8. Carefully add **25 µl** of the acetylation reagent to all acetylation tubes containing 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 **100 µl** aliquots from all acetylation tubes into the corresponding polypropylene or polystyrene assay tubes.
10. Pipette **100 µl** of antiserum into all assay tubes except the TC.
11. Vortex mix all assay tubes thoroughly. Cover the tubes, for example with plastic and incubate for 1 hour at room temperature (15–30°C).
12. Pipette **100 µl** of [^{125}I] cGMP into all assay tubes. The TC should be stoppered and set aside for counting.
13. Vortex mix all assay tubes thoroughly. Cover the tubes and incubate for between 15 and 18 hours at 2–8°C.

Day 2

14. Gently shake and swirl the bottle of separating second antibody reagent (blue-green) to ensure a homogenous suspension. Add **500 µl** to each tube except TC.
15. Vortex mix all tubes thoroughly and incubate for 10 minutes at room temperature (15–30°C).
16. Separate the antibody bound fraction by using either magnetic separation or centrifugation, as described below.

Magnetic separation

Attach the rack on to the separating reagent separator base and ensure that all tubes are in contact with the base plate. Leave for 15 minutes. After separation, do not remove the rack from the separator base. Pour off and discard the supernatant liquids. Keeping the separator inverted, place the tubes on a pad of absorbent tissues and allow to drain for 5 minutes.

Centrifugation

Centrifuge all tubes for 10 minutes at 1500 g or greater. After centrifugation, place the tubes carefully into suitable decantation racks then pour off and discard the supernatant liquids. Keeping the tubes inverted, place them on a pad of absorbent tissues and allow to drain for 5 minutes.
17. On completion of either the magnetic or centrifugal separation, firmly blot the rims of the tubes on the tissue pad to remove any adhering droplets of liquid. Do not re-invert the tubes once they have been turned upright.
18. Determine the radioactivity present in each tube by counting for at least 60 seconds in a gamma scintillation counter.

Note: Less sensitive curves can be obtained by carrying out this procedure without the delayed addition of assay tracer or without acetylation. Although not described these protocols are outlines in table 2.

Table 1. Radioimmunoassay protocol, Pipetting Guide (all volumes are in microlitres)

Tubes	TC	Bo	Stan - dard	Sam- ple
Buffer	-	**100**	-	-
Stan- dard	-	-	**100**	-
Sample	-	-		**100**
Anti- serum	-	**100**	**100**	**100**
Vortex mix, cover tubes and incubate for 1 hour at room temperature (15-30°C)				
Tracer	**100**	**100**	**100**	**100**
Vortex mix and incubate for 15-18 hours at 2-8°C				
Separa ting reagent	-	**500**	**500**	**500**
Vortex mix, incubate for 10 minutes at room temperature. Separate either using magnetic separator for 15 minutes or by centrifugation for 10 minutes at >1500 xg. Decant supernatants, drain for 5 minutes and count.				

Table 2. Alternative acetylation or non-acetylation protocol, Pipetting Guide (all volumes are in microlitres)

Tubes	TC	Bo	Stan - dard	Sam- ple
Buffer	**-**	**100**	**-**	**-**
Stan- dard	-	**-**	**100**	**-**
Sample	-	**-**		**100**
Anti- serum	-	**100**	**100**	**100**
Tracer	**100**	**100**	**100**	**100**
Vortex mix, cover tubes and incubate for 15-18 hours at 2-8°C				
Separa ting reagent	-	**500**	**500**	**500**
Separate asdescribed above.				

Calculation of results

The calculation is illustrated using representative data.The assay data collected should be similar to the data shown in table 3.

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

2. Calculate the percent Bo/TC using the following equation:

$$Bo/TC(\%) = \frac{Bo\ (cpm)}{TC\ (cpm)} \times 100$$

If the counter background is high, it should be subtracted from all the counts.

3. Calculate the percent bound for each standard and sample using the following equation:

$$B/Bo(\%) = \frac{Standard\ or\ sample\ (cpm)}{Bo\ (cpm)} \times 100$$

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

Plot B/Bo(%) (y axis) against the fmol standard per tube (x axis). The fmol per tube value of the samples can be read directly from the graph.
NSB is not normally determined, and is given for information only.

Table 3. Typical assay data for acetilation protocol

Tube	Conc. (fmol/ tube)	Avarage counts/ minute (cpm)	B/TC (%)	B/Bo (%)
TC	-	14133	-	-
NSB	-	283	2.0	-
Bo	-	6294	44.5	-
S1	2	5745	-	91.3
S2	4	5449	-	86.6
S3	8	4604	-	73.1
S4	16	3808	-	60.5
S5	32	2637	-	41.9
S6	64	1765	-	28.0
S7	128	1236	-	19.6

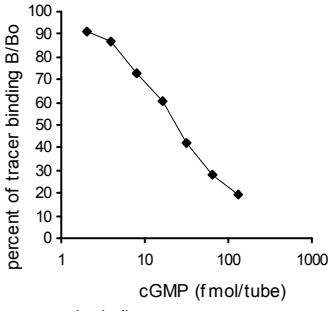


Figure 1: A typical standard curve (acetylation assay) (*Do not use to calculate unknown samples!)*

Characterization of assay

Magnetic and centrifugal separation methods yield identical assay performance and results.

Stability

The components of this radioimmunoassay system will have a shelf life of at least 4 weeks from the date of despatch.
Upon arrival, all components should be stored at 2–8°C where they are stable until the expiry date printed on the end pack label.

Non-specific binding

The non-specific binding (NSB) defined as the proportion of tracer bound in the absence of antibody was determined to be 2.1%. The NSB was independent of tracer batch and did not change over a 14-week storage period.

Sensitivity

The sensitivity, defined as the amount of cGMP needed to reduce zero dose binding by two standard deviations was 0.5 fmol.

Specificity

The antiserum cross-reactivity with related and other important compounds was determined by the 50% displacement technique. Values for both acetylation and non-acetylation assay systems are shown below:

Compound	(%) Cross-reactivity (acetylation)	(%) Cross-reactivity (non-acetylation)
cGMP	100	100
cAMP	<0.001	<0.001
AMP	<0.001	<0.001
ADP	<0.001	<0.001
ATP	<0.001	<0.001
GMP	<0.001	<0.001
GDP	<0.001	0.0075
GTP	<0.001	<0.001

Note: The displacement curve of cross-reacting substances is often not parallel to that of the standard ligand. This means that the interference will not be the same at all concentrations of the unknown. Reported cross-reactivities are meant to give only a general idea of possible inaccuracy due to lack of specificity of the antibody.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precautions

Radioactivity
This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Chemical hazard
Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 113 mg.

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 sheet for specific advice).

Safety data sheets

1. Product name:
Sodium azide
CAS No. 26628-22-8
R: 22-32 Toxic 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:
Sodium azide solution.
Hazards identification:
Toxic 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 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.

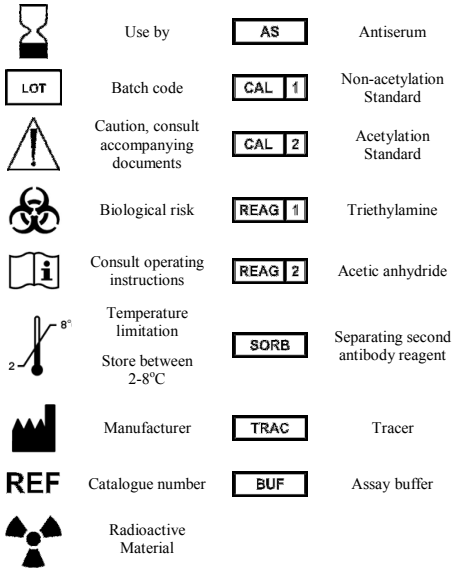
Toxicological information:
LD50: 27 mg/kg oral, rat
LD50: 20 mg/kg skin, rabbit
Ecological information:
Not applicable
Disposal consideration:
Up to 5 vials worth of material may be disposed of directly down the sink with water. If 6 or more vials are to be disposed of they should pass through a chemical waste route.
Note: Inorganic azides will react with lead and copper plumbing fixtures to give explosive residues. Disposal of significant quantities of azides via such plumbing is not recommended.
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.

2. 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 eyes. Irritating to the respiratory system. Harmful it swallowed, inhaled or absorved through the skin. Causes burns.
First aid measures:
In case of concact, immediately flush eyes or skin with copiousamounts 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 of all sources ignition. Wear suitable laboratory protective equiqment; 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,safetyglasses 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: 54.0 mm (20 °C). Lower explosion limit: 1.2. Upper explosion limit: 8.0. Flashpoint: 20°F. Formula weight: 101.19. Density: 0.726. Vapour density: 3.5.
Stability and reactivity:
Incompatible with acids and oxidizing agents. Therral 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:50ppm/30d-I sev.
LD50: 460 mg/kg oral, rat
LD50: 570 mg/kg skin, rabbit
LD50: 6 g/m3/2 h inh., mus
Ecological information:
Not applicable
Disposal consideration:
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.

3. 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 of the reach of children). In case of contact with eyes, rinse immediately with plenty of water of seek medicial 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 it swallowed, inhaled or absorved through the skin.Matrial is destructive to the tissues of the mucous membranes, upper respiratory tract, eyes and skin.
First aid measures:
In case of concact, immediately flush eyes or skin with copious 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 equiqment; 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 pickup 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. Wash thoroughly after handling. Keep tightly closed. Keep away from heat, sparks and open flame. Store ina 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: 4.6 mm (20 °C). Lower explosion limit: 2.8. Upper explosion limit: 10.3. Flashpoint: 130°F. Formula weight: 102.09. Density: 1.080. Vapour density: 3.52. Auto ignition temperature: 629°F.
Stability and reactivity:
Acids, bases, moisture, alcohols, oxidizing agents, reducing agents and finely powdered metals.
Toxicological information:
Skin-rabbit: 10 mg/24 h open mld. Skin-rabbit: 540 mg open mld. Eye-rabit: 250 microgramm open sev.
Ecological information:
Not applicable
Disposal consideration:
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.



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