GE Healthcare

Amersham 6-Keto-Prostaglandin $F_{1\alpha}$ [^{125}I] Biotrak Assay System with Magnetic Separation

100 tubes

Product Booklet

Code: RPA515

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1. Legal

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2. Handling

2.1. 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 the local regulations or codes of practice which relate to such matters.

Warning: Contains azide. See safety data sheet on p.28.

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 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).

2.2. Storage

Store the reagents at 2–8°C.

2.3. Expiry

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

3. Components of the assay system

The pack contains the following assay components, sufficient material for 100 tubes. All components for this kit should be stored at 2–8°C.

Standard
6-keto-prostaglandin $F_{1\alpha}$ standard, 8 ng lyophilised, containing 0.1% (w/v) azide. See safety data sheet on page 28.

Tracer
6-keto-prostaglandin $F_{1\alpha}$ [^{125}I]iodotyrosine methyl ester, ~74 kBq, 2µCi, in ethanol:water (1:1).

Antiserum
6-keto-prostaglandin $F_{1\alpha}$ antiserum, lyophilised, contains 0.08% azide.

Assay buffer
Phosphate buffer concentrate, which on dilution yields 0.05 M buffer, pH 7.3 containing 0.05% BSA and 0.1% sodium azide.

Amerlex-M second antibody reagent
Donkey anti-rabbit serum coated on to magnetisable polymer particles, with sodium azide, colour coded blue green, 55 ml. Ready for use.

4. Description

The Biotrak™ iodinated 6-keto-prostaglandin $F_{1\alpha}$ assay system from GE Healthcare has been specifically designed for research purposes.

The system combines the use of an iodinated 6-keto-prostaglandin $F_{1\alpha}$ tracer and an antiserum which is specific for 6-keto-prostaglandin $F_{1\alpha}$. This provides a rapid, simple and sensitive method for the determination of 6-keto-prostaglandin $F_{1\alpha}$ *in vitro* over the range 3–400 pg/tube.

The assay is based on the competition between unlabelled 6-keto-prostaglandin $F_{1\alpha}$ and a fixed quantity of the iodinated compound for binding to a protein which has a high specificity and affinity for 6-keto-prostaglandin $F_{1\alpha}$. With fixed amounts of antibody and radioactive ligand, the amount of radioactive ligand bound by the antibody will be inversely proportional to the concentration of added non-radioactive ligand (figure 1).

The antibody bound 6-keto-prostaglandin $F_{1\alpha}$ is then reacted with the Amerlex™-M second antibody reagent which contains second antibody that is bound to magnetisable polymer particles. Separation of the antibody bound fraction is effected by either magnetic separation or centrifugation of the Amerlex-M suspension and decantation of the supernatant.

Measurement of the radioactivity in the pellet enables the amount of labelled 6-keto-prostaglandin $F_{1\alpha}$ in the bound fraction to be calculated. The concentration of unlabelled 6-keto-prostaglandin $F_{1\alpha}$ in the sample is then determined by interpolation from a standard curve.

Free **Bound**

$[^{125}I]$6-keto-prostaglandin $F_{1\alpha}$ $[^{125}I]$6-keto-prostaglandin $F_{1\alpha}$-antibody

+ antibody

6-keto-prostaglandin $F_{1\alpha}$ 6-keto-prostaglandin $F_{1\alpha}$-antibody

Each pack contains sufficient material for 100 assay tubes permitting the construction of one standard curve and assay of 39 unknowns in duplicate.

5. Critical parameters

The following points are critical.

- Working standards should be prepared within one hour of performing the radioimmunoassay so as to minimise any effect of 6-keto-prostaglandin $F_{1\alpha}$ adsorption to the walls of the test tubes.
- Either distilled or deionised water may be used for reagent preparation, reconstituted components should be stored at 2–8°C and may be re-used within 7 days of dilution.

6. Additional materials and equipment required

The following materials and equipment are required but not supplied:

- Gamma counter
- Refrigerator
- Ice-bath
- Vortex mixer
- Magnetic stirrer and stir bar
- Test tube rack
- Disposable polypropylene test tubes (12 x 75 mm)
- Distilled or deionised water
- Pipettes or pipetting equipment with polypropylene tips, (100, 200, 300, 400, 500 and 1000 µl)
- Glass measuring cylinders (10 and 100 ml).
- Amerlex-M separators of comprising a magnetic base and assay rack, are available from Ortho-Clinical Diagnostics. These are for use in the magnetic protocol.
- Additional packs of 4 assay racks, are also available from Ortho-Clinical Diagnostics.
- Disposable polypropylene tubes are supplied by Sarstedt International, Rommelsdore, 5223 Numbrecht, Germany.

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

- Decantation racks
- Refrigerated centrifuge capable of 1500 x g

7. Specimen collection and sample preparation

Collect the blood in a tube with an anticoagulant, centrifuge the blood immediately and rapidly freeze the plasma. If blood samples cannot be rapidly processed, then the addition of indomethacin or aspirin to the anticoagulant is recommended. Either of these compounds will inhibit the subsequent metabolism of arachidonic acid to prostaglandins. One effective method is to collect the blood in a tube containing EDTA and indomethacin. Mixing 0.95 ml of an EDTA solution (2 g disodium EDTA and 0.8 g NaCl adjusted to pH 7.4 with NaOH and made up to a final volume of 100 ml in distilled water) with 0.05 ml of a 0.04 M indomethacin solution (50 mg indomethacin dissolved in 3.5 ml absolute ethanol) is recommended for the treatment of 10 ml of blood. The EDTA solution must be vortex mixed while the indomethacin is being added or the indomethacin will precipitate. Store the plasma samples at -15°C to -30°C, or below, until the assay is conducted.

Further information describing prostaglandin radioimmunoassays is contained in three relevant articles (8,9,10). It is well established that non-esterified fatty acids can interfere with the assays (3,11,12). If samples contain these interfering compounds, several methods are available for separating the prostaglandins from the fatty acids (13–16). In addition, Dray has evaluated blood drawing procedures (16).

Solid phase extraction procedures have become the method of choice for many researchers, giving high recovery and clean samples. However, liquid phase extraction techniques are still used.

Representative procedures for the extraction of 6-keto-prostaglandin $F_{1\alpha}$ from plasma using Amprep™ minicolumns and by solvent extraction are described below. This information is provided for

guidance only. It remains the investigators' responsibility to validate their chosen procedure with their own samples.

Amprep extraction

1) Column type

Amprep C2 100 mg code RPN1903

2) Sorbent conditioning

Rinse the column with 2 ml methanol
Rinse the column with 2 ml water

3) Sample treatment

Plasma-Acidify 1 ml plasma to pH 3, apply to the column

4) Interference elution

Wash column with 5 ml water
Wash column with 5 ml 10% ethanol
Wash column with 5 ml hexane (or petroleum ether 30–40°C)

5) Elution

Elute 6-keto-prostaglandin $F_{1\alpha}$ with 5 ml methyl formate

Notes

- The methyl formate should be dried under nitrogen or vacuum, and the extract redissolved in assay buffer before estimation.
- The typical recovery of [^{3}H]6-keto-prostaglandin $F_{1\alpha}$ is 90%.
- For further details of the Amprep range of products see your GE Healthcare representative.

Liquid phase (17)

1. Add tritiated 6-keto-prostaglandin $F_{1\alpha}$ to 1 ml biological sample for estimation of recovery.

2. Add 2 ml acetone and shake for 2 minutes.

3. Centrifuge at 4°C.

4. Transfer the supernatant to a separate tube and add 2ml hexane or petroleum ether.

5. Shake for 2 minutes and centrifuge at 4°C.
6. Discard the upper hexane layer.
7. Adjust the pH of the lower layer to 3.0–4.0 with 1 M citric acid.
8. Add 2 ml chloroform and shake for 2 minutes.
9. Centrifuge at 4°C.
10. The lower chloroform layer contains the extracted 6-keto-prostaglandin $F_{1\alpha}$ Separate and re-extract the top layer with 2 ml chloroform.
11. Combine the chloroform extracts, dry under nitrogen or vacuum and estimate the 6-keto-prostaglandin $F_{1\alpha}$ recovered.

8. Assay protocol

8.1. Reagent preparation

Storage

Either distilled or deionised water may be used for reagent preparation. Reconstituted components should be stored at 2–8°C and may be re-used within seven days of dilution.

Standard

Reconstitute the lyophilised 6-keto-prostaglandin $F_{1\alpha}$ standard by adding exactly 1.0 ml of distilled water. The contents of the vial should be mixed thoroughly to ensure that the lyophilised material is fully dissolved. The resulting solution contains 8 ng of 6-keto-prostaglandin $F_{1\alpha}$ per ml with 0.1% azide. A scheme for the preparation of working standards is shown below.

Assay buffer

Warm the assay buffer concentrate to room temperature and transfer the contents of the vial to a 100 ml graduated cylinder by repeated washing with distilled water. Adjust the final volume to 100 ml with distilled water, and mix thoroughly. The diluted assay buffer contains 0.05 M phosphate buffer, pH 7.3 with 0.05% BSA and 0.1% azide.

[^{125}]6-keto-prostaglandin $F_{1\alpha}$ tracer

The vial contains 6-keto-prostaglandin $F_{1\alpha}$[^{125}]iodotyrosine methyl ester in ethanol:water (1:1). Dilute with 10 ml of assay buffer and vortex mix thoroughly. The resulting solution contains approximately 74 kBq, 2 µCi of tracer in 0.05 M phosphate buffer, pH 6.8, with 0.05% BSA.

Antiserum

Reconstitute the lyophilised antiserum by adding 10 ml of distilled

water to the contents of the vial. Mix carefully to avoid foaming. The resulting solution contains 0.08% azide.

8.2. Preparation of working standards

Allow the reconstituted assay standard (8 ng/ml) to reach room temperature. This standard serves as the stock solution to make a series of standards by serial dilution performed as follows:

1. Label 8 polypropylene tubes, 3.1 pg, 6.2 pg, 12.5 pg, 25 pg, 50 pg, 100 pg, 200 pg and 400 pg, and pipette 500 µl of assay buffer into each tube.
2. Pipette 500 µl of the stock standard into the tube marked 400 pg and mix thoroughly.
3. Transfer 500 µl from tube 400 pg to tube 200 pg and mix again.
4. Repeat this doubling dilution successively with the remaining tubes.
5. 100 µl aliquots of each serial dilution will give rise to 8 standard levels of 6-keto-prostaglandin $F_{1\alpha}$ ranging from 3.1 to 400 pg.

Note: Working standards should be prepared within one hour of performing the radioimmunoassay so as to minimise any effect of 6-keto-prostaglandin $F_{1\alpha}$ adsorption to the walls of the test tubes.

8.3. Assay procedure

The procedure is summarised in table 1.

Day 1

1. Prepare all reagents as described in the previous sections. Standards should be freshly diluted immediately prior to performing the assay.
2. Equilibrate all reagents to room temperature, and mix before use.

3. Label polypropylene tubes (12 x 75 mm) in duplicate for total count tubes (TC), non-specific binding tubes (NSB), zero standard tubes (B_0) standards and samples.
4. Pipette 300 µl of assay buffer into the zero standard tubes (B_0) (see Table 1).
5. Pipette 400 µl of assay buffer into the non-specific binding tubes (NSB).
6. Pipette 200 µl of assay buffer into all standard and sample tubes.
7. Starting with the most dilute, pipette 100 µl of each diluted standard into the appropriately labelled tubes and vortex mix.
8. Pipette 100 µl of each sample, in duplicate, into the appropriately labelled tubes and vortex mix.
9. Pipette 100 µl of [^{125}I]6-keto-prostaglandin $F_{1\alpha}$ into all tubes and vortex mix.
10. Pipette 100 µl of antiserum into all tubes except the total count tubes (TC) and the non-specific binding tubes (NSB). The total count tubes (TC) should be stoppered and put aside for counting.

Note: All tubes except the total count tubes (TC) should now contain a total volume of 500 µl.

11. Vortex mix all tubes thoroughly for 2–5 seconds and incubate overnight (between 15 and 18 hours) at 2–8°C.

Day 2

12. Gently shake and swirl the bottle contain Amerlex-M second antibody reagent (blue-green) to ensure a homogeneous suspension. Then add 500 µl to each tube except the TC.
13. Vortex mix all tubes thoroughly and incubate for 10 minutes at room temperature (15–30°C).
14. Separate the antibody bound fraction by using either magnetic separation or centrifugation, as described overleaf.

Magnetic separation

Attach the rack on to the Amerlex-M separator base and ensure that all the 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 x 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.

15. On completion of either magnetic or centrifugal separation, firmly blot the rims of the inverted tubes on the tissues pad to remove any adhering droplets of liquid. Do not re-invert the tubes once they have been turned upright.

16. Determine the radioactivity present in each tube by counting for at least 60 seconds in a gamma scintillation counter.

Table 1 Radioimmunoassay protocol (All volumes are in microlitres)

Tube	Total Count (TC)	Non-specific binding (NSB)	Zero standard (B_0)	Standards	Samples
Buffer	-	400	300	200	200
Standards	-	-	-	100	-
Samples	-	-	-	-	100
[^{125}I]6-keto-prostaglandin $F_{1\alpha}$	100	100	100	100	100
Antiserum	-	-	100	100	100
Mix and incubate for 15–18 hours at 2–8°C					
Amerlex-M second antibody	-	500	500	500	500
Vortex mix. Incubate for 10 minutes at room temperature. Separate either using Amerlex-M separator for 15 minutes or by centrifugation for 10 minutes at ≥1500 x g. Decant supernatants, drain for 5 minutes and count.					

9. Data processing

9.1. Calculation of standard curve data

The calculation is illustrated using representative data.

The assay data you have collected should be similar to the data shown in table 1.

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

2. Calculate the percent B_0/TC by using the following equation:

$$\%B_0/TC = \frac{(B_0 \text{ cpm-NSB cpm})}{(TC \text{ cpm - background cpm})} \times 100$$

3. Calculate the percent bound for each standard and sample by 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 bound as a function of the $\log_{10}$ 6-keto-prostaglandin $F_{1\alpha}$ concentration.

Plot $\%B/B_0$ (y axis) against pg standard per tube (x axis) as shown in figure 2. The pg/tube value of the samples can then be read directly from the graph.

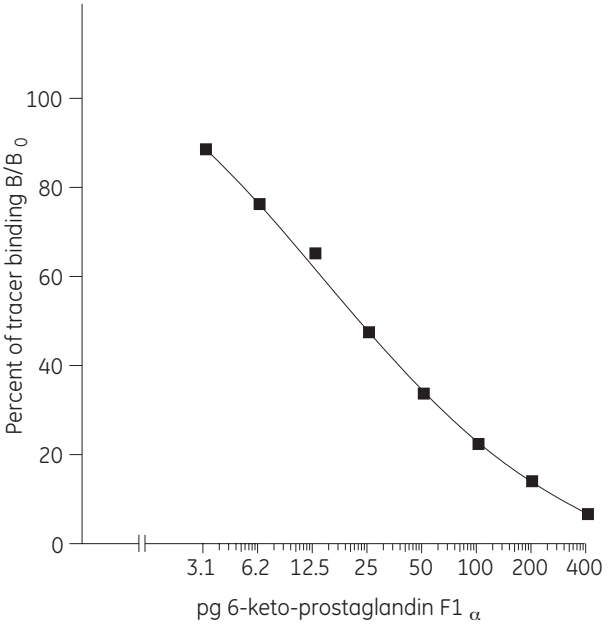

Figure 2. Prostaglandin $F_{2\alpha}$ standard curve

Table 1. Standard curve calculation using representative data

Tube	Mean cpm	Mean cpm-NSB	% $\frac{\text{B-NSB}}{\text{TC}}$	$\frac{\text{B-NSB} \times 100}{\text{B}_0\text{-NSB}}$
Total counts (TC)	33058	-	-	-
Non-specific binding (NSB)	1048	-	-	-
Zero standard (B_0)	15352	14304	43.4	-
Standard 3.1 pg/tube	13929	12881	-	88.6
Standard 6.2 pg/tube	11927	10879	-	76.1
Standard 12.5 pg/tube	10397	9349	-	65.4
Standard 25 pg/tube	7853	6805	-	47.6
Standard 50 pg/tube	5905	4857	-	33.9
Standard 100 pg/tube	4276	3228	-	22.6
Standard 200 pg/tube	3159	2111	-	14.7
Standard 400 pg/tube	2039	991	-	6.9
Background radiation	15	-	-	-

10. Additional information

10.1. Specificity

The specificity data for the 6-keto-prostaglandin $F_{1\alpha}$ antiserum is shown below:

Prostaglandins	% Cross-reactivity (50% B/B_0 displacement)
6-keto-prostaglandin $F_{1\alpha}$	100
Prostaglandin $F_{1\alpha}$	1.0
Prostaglandin $F_{2\alpha}$	0.8
Prostaglandin E_1	0.5
Prostaglandin E_2	0.5
Thromboxane B_2	<0.3

10.2. Amerlex-M accessories

Amerlex-M separator
Amerlex-M multivortexer
Amerlex-M racks (4 in a pack)

These products are available from Ortho-Clinical Diagnostics, or their assigned distributors.

10.3. Related products

Biotrak eicosanoid assay range

Thromboxane B_2	EIA	RPN 220
Thromboxane B_2, [^{125}I]	RIA	RPA 516
Leukotriene B_4	EIA	RPN 223
Leukotriene B_4, [^{3}H]	RIA	TRK 940
Leukotriene $C_4/D_4/E_4$	EIA	RPN 224
Platelet activating factor (PAF), [^{3}H]	SPA	TRK 990

Prostaglandin E_2	EIA	RPN 222
Prostaglandin E_2, [^{125}I]	RIA/AM	RPA 530
6-Keto-prostaglandin $F_{1\alpha}$	EIA	RPN 221
Prostaglandin $F_{2\alpha}$, [^{3}H]	RIA	TRK 900

Key:
RIA Radioimmunoassay
SPA Scintillation proximity assay
AM Amerlex-M
EIA Enzymeimmunoassay

For further details of GE Healthcare's scintillation proximity assay reagents and systems for homogeneous radioimunoassay see your GE Healthcare representative.

11. Background and references

A diverse array of mammalian cells and tissues enzymatically oxidise arachidonic acid to physiologically active compounds. These compounds include prostacyclin, thromboxanes, prostaglandins and leukotrienes.

Prostacyclin, also known as prostaglandin I_2 (PGI_2), is an unstable vinyl ether formed from the prostaglandin endoperoxide, prostaglandin H_2. This conversion of PGH_2 to prostacyclin is catalysed by prostacyclin synthetase and the two primary sites of synthesis are the veins and arteries. Prostacyclin has biological properties opposing the effect of thromboxane A_2. Prostacyclin is a vasodilator and a potent inhibitor of platelet aggregation (1) while thromboxane A_2 is a vasoconstrictor and a promoter of platelet aggregation (2,3). A physiological balance between the activities of these two effectors is probably important to maintaining a healthy vascular bed.

Prostacyclin is unstable and it undergoes a spontaneous hydrolysis to 6-keto-prostaglandin $F_{1\alpha}$.

Study of this reaction *in vitro* established that prostacyclin has a half-life of about 3 minutes (4). This half-life increases to 9–23 minutes in platelet-poor plasma (5). Due to this spontaneous hydrolysis of prostacyclin, the quantitation of 6-keto-prostaglandin $F_{1\alpha}$ is accepted by many researchers as a measure of prostacyclin formation (6).

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Safety data sheet SDS201/AD

Date of issue September 2006

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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: 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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