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# Instructions for ase nigh sensitiv

Instructions for use
3-CAT high sensitive ELISA

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use only – Not for use in diagnostic

### 3-CAT high sensitive ELISA

### 1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine. Flexible test system for various biological sample types and volumes.

Adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine are extracted by using a cis-diolspecific affinity gel, acylated and then converted enzymatically.

The competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized standards, controls and samples and the solid phase bound analytes compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigenantibody complexes are removed by washing. The antibody bound to the solid phase is detected by an antirabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.

Quantification of unknown samples is achieved by comparing their absorbance with a standard curve prepared with known standard concentrations.

## 2. Procedural Cautions, Guidelines and Warnings

- (1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- (2) The principles of Good Laboratory Practice (GLP) have to be followed.
- (3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable latex gloves and protective glasses where necessary.
- (4) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- (5) For dilution or reconstitution purposes, use deionized, distilled, of ultra-pure water.
- (6) The microplate contains snap-off strips. Unused wells must be stored at 2 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.X
- (7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (8) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- (9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (11) A standard curve must be established for each run.
- (12) The controls should be included in each run and fall within established confidence limits. The confidence
- limits are listed in the QC-Report (13) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
- (14) Avoid contact with Stop Solution containing 0.25 M H<sub>2</sub>SO<sub>4</sub>. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
- (15) TMB substrate has an writant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
- (16) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon
- (17) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.
- (18) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be ised for a test run. They must be stored properly until the manufacturer decides what to do with them. If It is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.

### 3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 2 months when stored at 2 - 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

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### 4. Materials

### 4.1 Content of the kit

**BA D-0032** Microtiter Plate - Ready to use

Content: 1 x 96 wells, empty in a resealable pouch

**BA D-0090** Adhesive Foil - Ready to use

Content: Adhesive Foils in a resealable pouch

Volume: 3 x 4 foils

**BA E-0030** WASH-CONC 50x Wash Buffer Concentrate - Concentrated 50x

Content:

Volume:

**BA E-0040** 

Content:

Volume:

**BA E-0055** 

SUBSTRATE
Substrate - Ready to use
Chromogenic substrate containing tetramethylbenzidine, substrate ouffer and hydrogen peroxide

3 x 12 ml/black vial, black cap

STOP-SOLN
Stop Solution - Ready to use

0.25 M sulfuric acid
3 x 12 ml/vial, light grey cap structions for Use P Content:

Volume:

**BA E-0080** 

Content:

Volume:

Hazards identification:

H290 May be corrosive to metals.

Adrenaline Microtiter Strips - Ready to use **BA E-0131** ADR MN

Content: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable blue pouch with

desiccant

**BA E-0231** Noradrenaline Microtiter Strips - Ready to use III NAD NMN

1 x 96 well (12x8) antigen precoated microwell plate in a resealable yellow pouch with Content:

desiccant

**BA E-0331** opamine Microtiter Strips - Ready to use DOP

 $1 \times 96$  well (12x8) antigen precoated microwell plate in a resealable green pouch with Content:

desiccant

**BA E-5110** Adrenaline Antiserum - Ready to use ADR-AS

Content: Rabbit anti-adrenaline antibody, blue coloured

Volume: 4 6 ml/vial, blue cap

BA E-52100 Noradrenaline Antiserum - Ready to use NAD-AS

Content: Rabbit anti-noradrenaline antibody, yellow coloured

Volume: 1 x 6 ml/vial, yellow cap

**BA E-5310 Dopamine Antiserum** - Ready to use

Content: Rabbit anti-dopamine antibody, green coloured

Volume: 1 x 6 ml/vial, dark green cap

**BA E-6612** Acylation Reagent - Ready to use ACYL-REAG

Content: Acylation reagent in DMSO Volume: 1 x 3 ml/vial, white cap

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Content: TRIS buffer

Volume: 1 x 4 ml/vial, green cap

**BA R-4617** TE-BUFF **TE Buffer** – Ready to use

Content: TRIS-EDTA buffer

Volume: 1 x 4 ml/vial, brown cap

Standards and Controls - Ready to use

Cat. no.	Component	Colour/		centrati ng/ml	ion		centrati nmol/l	on	Volume/
	·	Сар	ADR	NAD	DOP	ADR	NAD	DOP	Vial
<b>BA R-5601</b>	STANDARD A	white	0	0	0	0	0	0	4 101
BA R-5602	STANDARD B	light yellow	0.5	0.2	0.5	2.7	1.2	3.3	4 ml
BA R-5603	STANDARD C	orange	1.5	0.6	1.5	8.2	3.5	9.8	M 4 ml
BA R-5604	STANDARD D	dark blue	5	2	5	27	12	793	4 ml
BA R-5605	STANDARD E	light grey	20	8	20	109	47	131	4 ml
BA R-5606	STANDARD F	black	80	32	80	437	189	522	4 ml
BA R-5651	CONTROL 1	light green	Refer to	o QC-Re	oort for e	expected	value ar	nd	4 ml
BA R-5652	CONTROL 2	dark red	accepta	able rang	je!		5		4 ml
Conversion:	Adrenaline (r	ng/ml) x 5.46	= Adrena	aline (nn	nol/l)	in			

Noradrenaline (ng/ml) x 5.91 = Noradrenaline (nmol/NO)

Dopamine  $(ng/mI) \times 6.53 = Dopamine (nmol/I)$ 

Content: Acidic buffer with non-mercury stabilizer, spiked with defined quantity of adrenaline,

noradrenaline, and dopamine

**BA R-6611** Acylation Buffer - Ready to use

Content: Buffer with light alkaline pH for the acylation

Volume: 1 x 20 ml/vial, white cap

**BA R-6614** Coenzyme Ready to use COENZYME

Content: S-adenosyl-L-methionine Volume: 1 x 4 ml/vial, purple cap

**BA R-6615** Enzyme – Lyophilized

Content: Catechol-O-methyltransferase

Volume: 4 vials, pink cap

**BA R-6618** Extraction Plate - Ready to use

2 x 48 well plates coated with boronate affinity gel in a resealable pouch Content:

**BA R-6619** Hydrochloric Acid - Ready to use

Content: 9.025 M Hydrochloric Acid, yellow coloured

Volume: 1 x 20 ml/vial, dark green cap

# 4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 1 750 µl; 1 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 650 nm
- Shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Temperature controlled incubator (37 °C) or similar heating device
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

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### 5. Sample collection and storage

Storage: up to 6 hours at 2 - 8 °C; for longer periods (up to 6 months) at -20 °C or -80 °C. Advice for the preservation of the biological sample: to prevent catecholamine degradation, add EDTA (final concentration 1 mM) and sodium metabisulfite (final concentration 4 mM) to the sample.

### 6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate measurements are recommended. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the Enzyme Immunoassay is between 20 - 25 °C.



riangle In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a hicroplate reader set to 405 nm

### 6.1 Preparation of reagents

### **Wash Buffer**

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1,000 ml.

Storage: 2 months at 2 - 8 °C

### **Enzyme Solution**

Reconstitute the content of the vial labelled 'Enzyme' with 1 ml water (deionized, distilled, or ultra-pure) and mix thoroughly. Add 0.3 ml of Coenzyme followed by 0.7 ml of Adjustment Buffer. The total volume of the Enzyme Solution is 2.0 ml.



The Enzyme Solution has to be prepared freshly prior to the assay (not longer than 10 - 15 minutes in advance). Discard after use!

# Adrenaline Microtiter Strips, Noradrenaline Microtiter Strips and Dopamine Microtiter Strips

In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

### **Acylation Reagent**

The Acylation Reagent (BA E-6612) has a freezing point of 18.5 °C. To ensure that the Acylation Reagent is liquid when being used, it must be ensured that the Acylation Reagent has reached room temperature and forms a homogeneous, crystal-free solution before being used.

# 6.2 Sample preparation

The 3-CAT high sensitive ELISA is a flexible test system for various biological sample types and volumes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to fit the protocol to his specific needs.

- Avoid excess of acid: excess of acid might exceed the buffer capacity of the extraction buffer. A pH > 7.0 during the extraction is mandatory.
- Prevent catecholamine degradation by adding preservatives to the sample (see Sample Collection and storage).
- Avoid chaotropic chemicals like perchloric acid. The high salt content might reduce the recovery of catecholamines. If your samples already contain high amounts of perchloric acid, neutralize them prior to the extraction step.
- Tissue samples can be homogenised in 0.01 N HCl in the presence of EDTA and sodium metabisulfite. Under these conditions, catecholamines are positively charged which reduces binding to proteins and optimizes solubility.
- Avoid samples that contain substances with a cis-diol structure. These will reduce the recovery of the catecholamines.
- It is advisable to perform a "Proof of Principle" to determine the recovery of the catecholamines in your samples. Prepare a stock solution of adrenaline, noradrenaline and dopamine. Add small amounts (to change the native sample matrix as less as possible) of the stock solutions to the sample matrix and check the recovery.
- The used sample volume determines the sensitivity of the test. Determine the sample volume needed to determine the catecholamines in your sample by testing different amounts of sample volume.

If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer or your local distributor directly!

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### 6.3 Extraction and acylation

The 3-CAT high sensitive ELISA offers a flexible test system for various biological sample types and volumes. Step 1 of the extraction procedure depends on the sample volume:

- in case you have sample volumes between 1 100 μl follow **1.1**
- in case you have sample volumes between 100 500 µl follow 1.2
- in case you have sample volumes between 500 750 µl follow 1.3

# riangle Within a run it is only possible to measure samples with the same volume!

### 1.2 1.3 1. Sample volume 100 - 500 µl Sample volume 1 - 100 µl Sample volume 500 - 750 µl Pipette into the respective wells Pipette into the respective wells Pipette into the respective wells of the Extraction Plate: of the Extraction Plate: of the Extraction Plate: 30 µl standards, 30 µl 30 µl standards, 30 µl 30 μl standards, 30 μl controls and 500 - 750 µl controls and 1 - 100 µl controls and 100 - 500 µl sample. sample. sample. Fill up each well with water Fill up each well with water Fill up each well with water (deionized, distilled, or ultra-(deionized, distilled, or ultra-(deionized, distilled, or ultra-pure) pure) to a **final volume** of to a **final volume** of 500 µl [e.g. pure) to a final volume of 100 µl [e.g. 30 µl standard plus 30 µl standard plus 470 µl water 750 µ [e.g. 30 µl standard plus 70 µl water (deionized, distilled, (deionized, distilled, or ultra-720 pl water (deionized, distilled, or ultra-pure)]. pure)]. or ultra-pure)]. 2. Pipette 25 µl of TE Buffer into all wells. Cover the plate with **Adhesive Foil**. Shake **60 min** at **RT** (20 – 25 ©) on a **shaker** (approx. 600 rpm). 3. Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material. 4. 5. Pipette 1 ml of Wash Buffer into all wells. 6. Shake **5 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). 7. Blot dry by tapping the inverted plate on absorbent material. Wash one more time as described (step 5, 6 and 7)! 8. Pipette 150 ul of Acviation Buffer into all wells. 9. Pipette 25 µl of Acylation Reagent into all wells. 10. 11. Shake **20 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). Empty the plate and blot dry by tapping the inverted plate on absorbent material. 12. 13. Pipette 1 ml of Wash Buffer into all wells.

- 14. Shake **5 min** at **RT** (20© 25 °C) on a **shaker** (approx. 600 rpm).
- Blot dry by tapping the inverted plate on absorbent material. 15.
- Wash one more time as described (step 13, 14, 15). 16.
- Pipette **200 µl** of **Hydrochloric Acid** into all wells. **17.**
- Cover plate with **Adhesive Foil**. Shake **10 min** at **RT** (20 25 °C) on a **shaker** (approx. 600 rpm). 18.
- Do not decant the supernatant thereafter!
  - 190 µl of the supernatant is needed for the subsequent enzymatic conversion

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### 6.4 Enzymatic Conversion

**Adrenaline** 

1.	Pipette <b>190</b> µl of the <b>extracted standards, controls</b> and <b>samples</b> into the respective wells of the <b>Microtiter Plate.</b>
2.	Add <b>50 µl</b> of <b>Enzyme Solution</b> (refer to 6.1) to all wells.
3.	Cover plate with <b>Adhesive Foil</b> . Shake <b>1 min</b> at <b>RT</b> (20 – 25 °C) on a <b>shaker</b> (approx. 600 rpm) to mix.
4.	Incubate for <b>2 h</b> at <b>37 °C</b> .  The following volumes of the supernatants are needed for the subsequent ELISA:

75 µl

75 µl

**Dopamine** 

### 6.5 Adrenaline, Noradrenaline and Dopamine ELISA

75 µl

1.	Pipette 75 $\mu$ I of standards, controls and samples from the Enzyme Plate (refer to	6.4) into the
	respective pre-coated <b>Microtiter Strips (*1)</b> .	KNO
2.	Pipette <b>50</b> $\mu$ I of the respective <b>Antiserum</b> (*2) into all wells.	Ni:

**Noradrenaline** 

- Cover the plate with Adhesive Foil. Shake 1 min at RT (20 25 °C) on a shaker (approx 600 rpm).
  Incubate for 15 20 h (overnight) at 2 8 °C.
- 5. Remove the foil. Discard or aspirate the content of the wells. Wash the plate 4 x by adding 300 μl of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
- **6.** Pipette **100** μ**I** of **Enzyme Conjugate** into all wells.
- **7.** Incubate **30 min** at **RT** (20 25 °C) on a **shaker** (approx. 600 rpm).
- 8. Discard or aspirate the content of the wells. Wash the plate 4 x by adding 300 μI of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
- 9. Pipette 100  $\mu l$  of Substrate into all wells.
- **10.** Incubate **20 30 min** at **RT** (20 25 °C) on a **shaker** (approx. 600 rpm).
- $\triangle$  Avoid exposure to direct sunlight!
- 11. Pipette 100 µl of Stop Solution into all wells
- **12. Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).
- $\overline{\mathbb{N}}$
- (\*1): Adrenaline Microtiter Strips Noradrenaline Microtiter Strips, Dopamine Microtiter Strips
- (\*2): Adrenaline Antiserum, Noradrenaline Antiserum, Dopamine Antiserum

### 7. Calculation of results

The standard curve from which the concentrations of the samples can be read off, is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).

Use a non-linear regression for curve fitting (e.g. 4-parameter, marquardt).

Correction factor =

This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.

The concentrations of the samples taken from the standard curve have to be multiplied by a correction factor.

30 µl (volume of standards extracted)
sample volume (µl) extracted

### Example

 $750~\mu l$  of the sample is extracted and the concentration taken from the standard curve is 0.15~ng/ml noradrenaline.

Correction factor = 30/750 = 0.04

Concentration of the sample =  $0.15 \text{ ng/ml} \times 0.04 = 0.006 \text{ ng/ml} = 6 \text{ pg/ml}$  noradrenaline

### Conversion

Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/l) Noradrenaline (ng/ml) x 5.91 = Noradrenaline (nmol/l) Dopamine (ng/ml) x 6.53 = Dopamine (nmol/l)

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# 7.1 Quality control

The confidence limits of the kit controls are indicated on the QC-Report.

# 8. Assay characteristics

	Substance	Cros	s Reactivity (	%)
		Noradrenaline	Adrenaline	Dopamine
	Derivatized Adrenaline	0.14	100	0.03
Analytical Specificity	Derivatized Noradrenaline	100	0.20	0.87
(Cross Reactivity)	Derivatized Dopamine	0.2	< 0.0007	100
	Metanephrine	< 0.003	0.64	< 0.007
	Normetanephrine	0.48	0.0009	0.008
	3-Methoxytyramine	< 0.003	< 0.0007	0.55
	3-Methoxy-4-hydroxyphenylglycol	0.01	0.03	0.007
	Tyramine	< 0.003	< 0.0007	0.13
	Phenylalanine, Caffeinic acid, L-		< 0.0007	< 0.007
	Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid		6914	

Sensitivity	Adrenaline	Noradrenaline Noradrenaline	Dopamine
(Limit of Detection)	0.25 ng/ml x C*	0.1 ng/ml x C*	0.25 ng/ml x C*

# C\* = Correction factor (refer to 7.)

fer to 7.)	, co *	
Adrenaline	Noradrenaline	Dopamine
10 pg/ml	4 pg/ml	10 pg/ml
	Adrenaline	Adrenaline Noradrenaline

Functional Sensitivity	Adrenaline	Noradrenaline	Dopamine
(750 µl undiluted sample)	15 pg/ml	6 pg/ml	15 pg/ml

Precision		L'U		
Intra-Assay Human EDT	A-Plasma	Si		
	Sample	Mean ± 3 SD (pg/ml)	SD (pg/ml)	CV (%)
	high	1,329.3 ± 372.6	124.2	9.3
Adrenaline	medium	412.1 ± 129.6	43.2	10.5
	low	37.9 ± 19.5	6.5	17.1
	high	1,377.4 ± 483.6	161.2	11.7
Noradrenaline	medium	502.6 ± 126.9	42.3	8.4
	low	32.7 ± 15.3	5.1	15.6
	high	1,438.6 ± 465.6	155.2	10.8
Dopamine	medium	565.9 ± 246.3	82.1	14.5
0	low	$56.4 \pm 36.3$	12.1	21.5

Intra-Assay Cell Culture	Medium (RPM	I)		
	Sample	Mean ± 3 SD (pg/ml)	SD (pg/ml)	CV (%)
01	high	1,649.6 ± 555.0	185	11.2
Adrenaline S	medium	526.2 ± 186.6	62.2	11.8
0,	low	38.7 ± 18.9	6.3	16.3
25	high	2,027.8 ± 712.5	237.5	11.7
Noradrenaline	medium	716.5 ± 179.7	59.9	8.4
<b>X</b>	low	46.0 ± 16.8	5.6	12.2
	high	2,784.5 ± 1,238.7	412.9	14.8
Dopamine	medium	1,003.7 ± 526.2	175.4	17.5
	low	74.7 ± 51.6	17.2	23.0

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Recovery	Mean (%)	Range (%)	SD (%)	CV (%)
Adrenaline				
Human EDTA-Plasma	104.0	89.4 - 128.3	13.1	12.6
Cell Culture Medium	95.5	81.6 – 109.6	8.3	8.7
Noradrenaline				
Human EDTA-Plasma	116.5	104.8 - 125.6	8.0	6.9
Cell Culture Medium	96.7	70.6 - 124.7	17.1	17.7
Dopamine				
Human EDTA-Plasma	97.7	83.7 - 115.9	11.8	12.1
Cell Culture Medium	98.6	77.7 - 113.4	12.1	12.2

Cell Culture Medium	96.7	70.6 – 124.7	17.1	17.7
Dopamine				
Human EDTA-Plasma	97.7	83.7 - 115.9	11.8	12.1
Cell Culture Medium	98.6	77.7 - 113.4	12.1	12.2
		please contact your lo	oruseprov	ded with the ki
ne nability of the mant nanufacturer takes no l he use of this product.	macturer Snam	be innited to the rep	iacement of d	erective products. The
nanufacturer takes no l he use of this product.	iability for any	be innited to the rep	iacement of d	ctly or indirectly fron
manufacturer takes no lithe use of this product.  mbols:	iability for any	damages or expense	es arising dire	ctly or indirectly from  Contains sufficient for
nanufacturer takes no lengthe use of this product.  nbols:  Storage temperature	re LOT	Manufacturer  Batch code	es arising dire	ctly or indirectly from  Contains sufficient for
manufacturer takes no lithe use of this product.  nbols:  Storage temperature  Use-by date  Consult instructions	re LOT	Manufacturer  Batch code	es arising dire	ctly or indirectly from  Contains sufficient for