Instructions for Use

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ACTH ELISA

Enzyme immunoassay use in the quantitative determination of human adrenocorticotropic hormone (ACTH) in EDTA-plasma.

REF **RE53081**

Σ **96**

2°C 8°C

EU: IVD CE

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INTENDED USE

Enzyme immunoassay use in the quantitative determination of human adrenocorticotropic hormone (ACTH) in EDTA-plasma. The test is useful for detecting elevated and deficient ACTH levels.

Indications for use:

Patient may have a higher than normal levels of ACTH with

1. Addison's disease or primary adrenal insufficiency
2. Congenital adrenal hyperplasia
3. Cushing's syndrome
4. Cushing's disease
5. Multiple endocrine neoplasia (MEN), type I

Patient may have a lower than normal levels of ACTH with

6. Hypopituitarism and/or secondary adrenal insufficiency
7. Adrenal gland tumor
8. Other tumors that produce cortisol

SUMMARY OF PHYSIOLOGY

ACTH is a 39 amino acid polypeptide with a molecular weight of 4540 Dalton. ACTH is secreted from corticotropes in the anterior lobe (or adenohypophysis) of the pituitary gland in response to corticotropin-releasing hormone (CRH) released by the hypothalamus. ACTH is synthesized from pre-pro-opiomelanocortin (pre-POMC). The removal of the signal peptide during translation produces the 241-amino acid polypeptide POMC, which undergoes a series of post-translational modifications such as phosphorylation and glycosylation before it is proteolytically cleaved by endopeptidases to yield various polypeptide fragments with varying physiological activity.

ACTH is an important component of the hypothalamic-pituitary-adrenal axis and is often produced in response to biological stress. It stimulates secretion of glucocorticoid steroid hormones from adrenal cortex cells especially in the zona fasciculata of the adrenal. ACTH acts by binding to cell surface ACTH receptors, which are located primarily on adrenocortical cells of the adrenal cortex.

ASSAY PRINCIPLE

This ELISA kit is designed, developed and produced for the quantitative measurement of human ACTH in EDTA-plasma sample. The assay utilizes the two-site "sandwich" technique with selected antibodies that bind to N-terminal and C-terminal epitopes of ACTH.

Assay standards, controls and patient samples are added directly to wells of a microtiter plate that is coated with antibody to the C-terminal of human ACTH. Immediately, a horseradish peroxidase (HRP) conjugated anti N-terminal of human ACTH antibody is added to each well. After the first incubation period, a "sandwich" of solid-phase polyclonal antibody - human ACTH – HRP conjugated monoclonal antibody" is formed. The unbound antibodies and buffer matrix are removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction, which is terminated with an acidic reagent (i.e. ELISA stop solution). The absorbance is then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the wall of each microtiter well is directly proportional to the amount of human ACTH in the test sample. A standard curve is generated by plotting the absorbance versus the respective human ACTH concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of human ACTH in test samples is determined directly from this standard curve.

REAGENTS: Preparation and Storage
This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.

1. Microtiter Plate **MTP**
One Microtiter Plate with twelve by eight strips (96 wells total) coated with polyclonal anti-human ACTH antibody. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

2. Enzyme Conjugate **ENZCONJ** **CONC**
One vial containing 0.25 mL of 21-fold concentrate HRP labeled anti-human ACTH antibody in a stabilized protein matrix. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. Enzyme Conjugate Diluent **ENZCONJDIL**
One vial containing 5 mL ready to use buffer. It should be used only for Enzyme Conjugate dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. Standards **CAL 1- 6** **LYO**
Six vials containing human ACTH in a lyophilized bovine serum based matrix with a non-azide preservative. **Refer to the vial for exact concentration of the standard.** These standards should be stored at 2 – 8°C and are stable until the expiration date on the kit box. Refer to assay procedure section for dilution direction.

5. Controls **CONTROL 1-2** **LYO**
Two vials containing human ACTH in a lyophilized bovine serum based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8°C and are stable until the expiration date on the kit box. Refer to assay procedure section for reconstitution instructions.

6. TMB Substrate Solution **TMB SUBS**
One bottle containing 25 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

7. TMB Stop Solution **TMB STOP**
One bottle containing 12 mL of stop solution. This reagent may be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

8. Wash Buffer **WASHBUF** **CONC**
One bottle containing 30 mL of 30-fold concentrate. Before use the contents must be diluted with **870 mL** of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

SAFETY PRECAUTIONS

The reagents must be used in professional laboratory. Source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potential infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 25 µL, 200 µL, etc.
2. Disposable pipette tips suitable for above volume dispensing.
3. Aluminum foil.
4. Deionized or distilled water.
5. Plastic microtiter well cover or polyethylene film.
6. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
7. Spectrophotometric microtiter Plate reader capable of reading absorbance at 450/650 or 450/620nm.

SPECIMEN COLLECTION

Since the circulating ACTH shows a 24 hours circadian rhythms, it is recommended to draw blood sample in the early morning, before 8 a.m. Patients should stop taking steroid drugs before drawing blood sample, at the consultation of their physician.

EDTA-plasma is a suitable specimen for human ACTH measurement. A total of 0.4 mL EDTA-plasma is required for duplicate determination of human ACTH with this test kit. Whole blood should be collected using lavender-top Vaccutainer and the plasma separated according to manufacturer's instruction. The EDTA-plasma should be separated from other cells right after or within one hour of blood collection. The plasma should be transferred to a clean test tube right after centrifugation. **Plasma samples should be stored at – 20ºC** if the assay is not to be performed within 3 hours. Avoid more than three times freeze-thaw cycles of specimen.

Samples of serum, heparin plasma and citrate plasma should not be used for ACTH measurement.

ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate must be diluted to working solution prior to use. Please see REAGENTS section for details.

(3) Reconstitute assay standards and controls by adding **2.0 mL** of deminerialized water to each standard and control bottle. Allow the standard and controls to sit undisturbed for 5 minutes, and then mix well by inversions or gentle vortexing. One must make sure that all solid is dissolved completely prior to use. These reconstituted standards and controls may be stored at 2- 8°C for up to 24 hours or below –10 °C for long-term storage. Do not exceed 3 freeze-thaw cycles.

(4) Prepare Enzyme Conjugate working solution by 1:21 fold dilution of the ACTH Enzyme Conjugate by adding the Enzyme Conjugate into the Enzyme Conjugate Diluent. Following is a table that outlines the relationship of strips used and antibody mixture prepared. **NOTE:** the Enzyme Conjugate should be prepared just prior to the beginning of the assay.

Dilution Scheme	Enzyme Conjugate Diluent	Enzyme Conjugate
1	0.4 mL	20 µL
2	0.8 mL	40 µL
3	1.2 mL	60 µL
4	1.6 mL	80 µL
5	2.0 mL	100 µL
6	2.4 mL	120 µL
7	2.8 mL	140 µL
8	3.2 mL	160 µL
9	3.6 mL	180 µL
10	4.0 mL	200 µL
11	4.4 mL	220 µL
12	4.8 mL	240 µL

(5) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3	STRIP 4
A	STD 1	STD 5	SAMPLE 1	SAMPLE 5
B	STD 1	STD 5	SAMPLE 1	SAMPLE 5
C	STD 2	STD 6	SAMPLE 2	SAMPLE 6
D	STD 2	STD 6	SAMPLE 2	SAMPLE 6
E	STD 3	C 1	SAMPLE 3	
F	STD 3	C 1	SAMPLE 3	
G	STD 4	C 2	SAMPLE 4	
H	STD 4	C 2	SAMPLE 4	

(6) Place a sufficient number of Anti-ACTH antibody coated microwell strips in a holder to run human ACTH standards, controls and unknown samples in duplicates.

2. Assay Procedure:

(1) Add **200 µL** of Standards, Controls and patient samples into the designated microwells.
(2) Immediately add **25 µL** of Enzyme Conjugate to each well.
(3) Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for **2 hr**. ± 5 minutes **at 400 to 450 rpm**.
(4) **Wash** each well **5 times** by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated Microtiter Plate washer can be used.
(5) Add **200 µL** of ELISA TMB Substrate into each of the wells.
(6) Cover the plate with aluminum foil or other material to avoid exposure to light. Incubate plate static, at room temperature for **20 minutes**.
(7) Immediately add **50 µL** of TMB Stop Solution into each of the wells. Mix gently.
(8) Read the absorbance at 450 nm with reference filter at 620 nm or 650 nm.

PROCEDURAL NOTES

1. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. An orbital mixer with a larger orbit radius (e.g. > 1 cm) may be used at speeds of 150 to 200 rpm.
7. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.
9. Adapting this assay to automated ELISA system such as DS-2 (Diamedix Corp., Miami), a procedural validation is necessary if there is any modification of the assay procedure.

INTERPRETATION OF RESULTS

It is recommended to use a point-to-point or 4-parameter standard curve fitting.

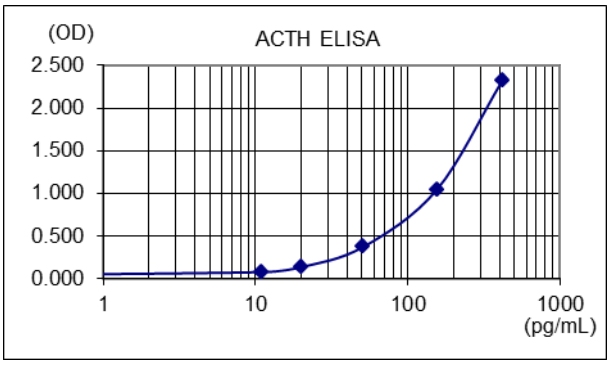
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the level 1 standard (0 pg/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The human ACTH concentrations for the controls and the patient samples are read directly from the standard curve using their respective corrected absorbance.

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from this EDTA plasma ACTH ELISA are represented. **This curve should not be used in lieu of standard curve generated with each assay.**

Well I.D.	OD 450/650 nm Absorbance			Results
	Readings	Average	Corrected	
Std-1: 0 pg/mL	0.025 0.029	0.027	0.000	
Std-2: 11 pg/mL	0.107 0.102	0.105	0.074	
Std-3: 20 pg/mL	0.160 0.160	0.160	0.133	
Std-4: 51 pg/mL	0.400 0.395	0.398	0.371	
Std-5: 155 pg/mL	1.057 1.081	1.069	1.042	
Std-6: 416 pg/mL	2.316 2.375	2.346	2.319	
Control 1	0.244 0.260	0.252	0.225	32.0 pg/mL
Control 2	0.676 0.714	0.695	0.668	97.1 pg/mL



EXPECTED VALUES

EDTA plasma samples from normal healthy adults ages 20 – 60 were collected and measured with this ELISA.

The recommended **normal range** for ACTH concentration by using this ELISA is between 1 - 72 pg/mL. We strongly recommend for each clinical laboratory to establish its own normal range by measuring EDTA plasma samples with this ELISA. Please note that sample collection time may have impact on the ACTH normal range.

LIMITATION OF THE PROCEDURE

1. This ACTH assay requires EDTA-plasma sample for testing. Serum sample may show a lower ACTH level and must not be used, because ACTH is not stable in serum.
2. Because of 24 hours circadian rhythms of circulating ACTH levels, the time of day the sample was collected should be considered when interpreting test results. Therefore, a normal ACTH test result doesn't rule out related diseases.
3. For sample values reading greater than highest standard, it is recommend to re-assay samples with dilution (i.e. 1:10 or 1:100 with standard zero).
4. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls.

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity (LLOD) of the ACTH ELISA as determined by the 95% confidence limit on 8 replicate determinations of zero standard is less than 1 pg/mL.

High Dose “hook” effect

This assay has showed that it did not have any high dose “hook” for ACTH levels up to 10,000 pg/mL.

Precision

The intra-assay precision was validated by measuring three control samples with 16 replicate determinations.

Sample #	Mean ACTH Value (pg/mL)	CV (%)
1	36.1	7.6
2	66.5	8.6
3	276.9	10.3

The inter-assay precision was validated by measuring two control levels in duplicate in 16 individual assays.

Sample #	Mean ACTH Value (pg/mL)	CV (%)
1	32.1	7.1
2	261.0	5.3

Linearity

Two ACTH standard levels were diluted with standard zero (i.e. level 1 standard) and tested. The results of ACTH percent recovery value in pg/mL are as follows:

DILUTION	OBSERVED VALUE (pg/mL)	RECOVERY (%)
Neat A	416	-
1:2	231.4	111
1:4	102.1	98
1:8	52.1	100
1:16	26.3	101
Neat B	155	-
1:2	80.3	104
1:4	38.6	100
1:8	20.5	106
1:16	9.3	96

Two EDTA plasma samples were collected, spiked with a high ACTH standard and tested. The results of ACTH percent recovery value in pg/mL are as follows:

DILUTION	OBSERVED VALUE (pg/mL)	RECOVERY (%)
Neat A	184.1	-
1:2	105.8	115
1:4	58.5	127
1:8	22.6	98
Neat B	33.3	-
1:2	17.1	103
1:4	9.4	113
1:8	5.1	121

Spike Recovery

Two EDTA plasma samples and three assay standards (45, 135 and 405 pg/mL) were combined at equal volumes and tested. The results are as follows:

DILUTION	OBSERVED VALUE (pg/mL)	EXPECTED VALUE (pg/mL)	RECOVERY (%)
Neat A	12.5	-	-
Std-4	25.1	28.8	87
Std-5	73.5	73.8	100
Std-6	254.0	208.8	122
Neat B	21.5	-	-
Std-4	26.1	33.3	79
Std-5	66.8	78.3	85
Std-6	208.1	213.3	98

Cross-Reactivity

Cross-reactivity was tested by spiking ACTH kit zero standards with concentrations of ACTH (1-17) fragments, ACTH (23-29) fragments, calcitonin hormone, osteocalcin protein, and parathyroid hormone (PTH). The results are provided below:

Cross-reactant	Spike Concentration (pg/ml)	% Cross-Reactivity
ACTH (1-17)	5 000	< 0.000
ACTH (23-29)	5 000	< 0.000
Calcitonin Hormone	5 000	+ 0.001
Osteocalcin protein	1 350	< 0.000
PTH (7-84)	5 000	< 0.000

Interference

Interference was tested by spiking EDTA plasma samples with concentrations of hemoglobin, lipid, and bilirubin. The results are provided below:

Bilirubin	**Interferent** *(Concentration Tested)*	**Test** (pg/mL)	**Control** (pg/mL)	**Bias** (d_{obs}) (%)
	20 mg/dL	39.82	37.65	+5.8%
Hemoglobin	**Interferent** *(Concentration Tested)*	**Test** (pg/mL)	**Control** (pg/mL)	**Bias** (d_{obs}) (%)
	40 mg/dL	54.6	52.8	+3.4%
	80 mg/dL	54.0	52.8	+2.3%
	160 mg/dL	59.6	52.8	+12.9%
Lipids	**Interferent** *(Concentration Tested)*	**Test** (pg/mL)	**Control** (pg/mL)	**Bias** (d_{obs}) (%)
	3000 mg/dL	56.5	50.1	+12.8%

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. IBL International DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall IBL International be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

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4. Kreek MJ, Wardlaw SL, Hartman N, Raghunath J, Friedman J, Schneider B, Frantz AG. Circadian rhythms and levels of beta-endorphin, ACTH, and cortisol during chronic methadone maintenance treatment in humans. Life Sci. 1983;33 Suppl 1:409-11

ACTH ELISA: Condensed Assay Protocol

1. **200 µL standards, controls and patient samples**

   +

   25 µL Enzyme Conjugate

   Incubate at room temperature for 2 hours on ELISA plate shaker
   Wash 5 x

   ↓

2. **200 µL TMB Substrate**

   Incubate at room temperature for 20 minutes static

   ↓

3. **50 µL TMB Stop Solution** *Immediately*

   ↓

4. **Read absorbance at 450/650 or 450/620 nm** *within 10 minutes*

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Store at: 2-8°C / Lagern bei: 2-8°C / Stocker à: 2-8°C / Almacene a: 2-8°C / Armazenar a: 2-8°C / Conservare a: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distribuidor: / Distributore: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
	Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.
For further country-specific classifications, please refer to the corresponding safety data sheet.

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