

Corticosterone (Human, Rat, Mouse) ELISA

Enzyme immunoassays for the quantitative determination of
Corticosterone in human serum or plasma.

REF

RE52211



12x8



2-8°C

EU: **IVD** **CE**



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Contents

| | | |
|----|--|---|
| 1 | INTRODUCTION..... | 2 |
| 2 | PRINCIPLE OF THE TEST | 2 |
| 3 | WARNINGS AND PRECAUTIONS..... | 2 |
| 4 | REAGENTS..... | 3 |
| 5 | SPECIMEN COLLECTION AND PREPARATION .. | 4 |
| 6 | ASSAY PROCEDURE..... | 5 |
| 7 | EXPECTED NORMAL VALUES | 6 |
| 8 | QUALITY CONTROL..... | 6 |
| 9 | PERFORMANCE CHARACTERISTICS | 7 |
| 10 | LIMITATIONS OF USE..... | 8 |
| 11 | LEGAL ASPECTS | 8 |
| 12 | REFERENCES / LITERATURE | 9 |

1 INTRODUCTION

1.1 Intended Use

The **IBL-International Corticosterone (Human, Rat, Mouse) ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of Corticosterone in serum and plasma.

1.2 Summary and Explanation

Corticosterone is a glucocorticoid secreted by the cortex of the adrenal gland. Corticosterone is produced in response to the stimulation of the adrenal cortex by adrenocorticotrophic hormone (ACTH) and is the precursor of aldosterone. Corticosterone is a major indicator of stress since stress increases the production of corticosteroids.

Studies involving corticosterone and levels of stress include impairment of long term memory retrieval¹, chronic corticosterone elevation due to dietary restrictions² and in response to burn injuries³. In addition to stress levels, corticosterone is believed to play a decisive role in sleep-wake patterns^{4,5}.

2 PRINCIPLE OF THE TEST

The IBL Corticosterone (Human, Rat, Mouse) ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on the Corticosterone molecule. Endogenous Corticosterone of a patient sample competes with a Corticosterone-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase conjugate is reverse proportional to the concentration of Corticosterone in the sample. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of Corticosterone in the patient sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for *in vitro* diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.

16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H₂SO₄. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant 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. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from IBL International.

4 REAGENTS

4.1 Reagents provided

1. **Microtiterwells**, 12 x 8 (break apart) strips, 96 wells;
Wells coated with an anti-Corticosterone antibody (polyclonal).
2. **Standard (Standard 0-6)**, 7 vials, 1 mL, ready to use;
Concentrations: 0, 5, 15, 30, 60, 120, 240 nmol/L
Conversion: 1 nmol/L = 34.646 ng/dL
 = 0.34646 ng/mL
Contain non-mercury preservative.
3. **Control Low & High**, 2 vials, 1 mL each, ready to use
For control values and ranges please refer to vial label or QC-Datasheet.
Contain non-mercury preservative.
4. **Enzyme Conjugate 250X Concentrate**, 1 vial, 150 µL;
Corticosterone conjugated to horseradish Peroxidase.
See „Reagent Preparation“.
5. **Conjugate Diluent**, 1 vial, 25 mL, ready to use.
6. **Substrate Solution**, 1 vial, 25 mL, ready to use;
Tetramethylbenzidine (TMB).
7. **Stop Solution**, 1 vial, 14 mL, ready to use;
Contains 0.5M H₂SO₄.
Avoid contact with the stop solution. It may cause skin irritations and burns.
8. **Wash Solution**, 1 vial, 30 mL (40X concentrated);
See “Reagent Preparation“.

Note: Additional *Standard 0* for sample dilution is available on request.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Wash Solution

Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Enzyme Conjugate

Dilute *Enzyme Conjugate* concentrate 1 + 250 in *Conjugate Diluent*.

This solution should be prepared freshly.

If the whole plate is used, dilute 100 µL *Enzyme Conjugate* with 25 mL *Conjugate Diluent*.

If the whole plate is not used at once prepare only the required quantity of *Enzyme Conjugate*.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, IBL has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Serum or EDTA plasma can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.

(E.g. Sarstedt Monovette for EDTA plasma)

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 24 hours at 2 °C to 8 °C prior to assaying.

Specimens held for a longer should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) Dilution 1:10: 10 µL Serum + 90 µL *Standard 0* (mix thoroughly)

b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature..

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **20 µL** of each *Standard, Control* and samples with new disposable tips into appropriate wells.
3. Dispense **200 µL Enzyme Conjugate** into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **60 minutes** at room temperature.
6. Briskly shake out the contents of the wells. Rinse the wells 3times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add **100 µL** of *Substrate Solution* to each well.
8. Incubate for **15 minutes** at room temperature.
9. Stop the enzymatic reaction by adding **50 µL** of *Stop Solution* to each well.
10. Read the OD at **450 ± 10 nm** with a microtiter plate reader **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 240 nmol/L. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay:

| Standard | Optical Units (450 nm) |
|-------------------------|------------------------|
| Standard 0 (0 nmol/L) | 2.31 |
| Standard 1 (5 nmol/L) | 1.69 |
| Standard 2 (15 nmol/L) | 1.35 |
| Standard 3 (30 nmol/L) | 1.10 |
| Standard 4 (60 nmol/L) | 0.87 |
| Standard 5 (120 nmol/L) | 0.63 |
| Standard 6 (240 nmol/L) | 0.48 |

7 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the IBL Corticosterone (Human, Rat, Mouse) ELISA the following values are observed:

| n | 5 % Percentile | 95 % Percentile |
|----|----------------|-----------------|
| 74 | 6.53 nmol/L | 31.09 nmol/L |
| | 226.24 ng/dL | 1077.14 ng/dL |

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or IBL directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 1.63 – 240 nmol/L.

9.2 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

| Component | Cross Reactivity |
|--------------------------|------------------|
| Corticosterone | 100 % |
| Progesterone | 7.4 % |
| Deoxycorticosterone | 3.4 % |
| 11-Dehydrocorticosterone | 1.6 % |
| Cortisol | 0.3 % |
| Pregnenolone | 0.3 % |
| other steroids | < 0.1 % |

9.3 Sensitivity

The analytical sensitivity of the IBL ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of *Standard 0* and was found to be < 1.631 nmol/L.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

| Sample | n | Mean (nmol/L) | CV (%) |
|--------|----|---------------|--------|
| 1 | 12 | 108.62 | 2.77 |
| 2 | 12 | 79.17 | 2.44 |
| 3 | 12 | 24.28 | 4.08 |

9.4.2 Inter Assay

The between assay variability is shown below:

| Sample | n | Mean (nmol/L) | CV (%) |
|--------|----|---------------|--------|
| 1 | 26 | 104.42 | 6.14 |
| 2 | 26 | 74.83 | 6.35 |
| 3 | 26 | 25.17 | 5.54 |

9.5 Recovery

Samples have been spiked by adding Corticosterone solutions with known concentrations.

The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

| Sample | Endogenous (nmol/L) | Added (nmol/L) | Measured Conc. (nmol/L) | Expected Conc (nmol/L) | Recovery (%) |
|------------|---------------------|----------------|-------------------------|------------------------|--------------|
| 1 Serum | 12.20 | 0.00 | 12.50 | | |
| | | 60.00 | 68.70 | 72.20 | 95.2 |
| | | 30.00 | 41.20 | 42.20 | 97.6 |
| | | 15.00 | 26.70 | 27.20 | 98.2 |
| 2 Serum | 42.20 | 0.00 | 40.50 | | |
| | | 60.00 | 99.10 | 102.20 | 97.0 |
| | | 30.00 | 69.10 | 72.20 | 95.7 |
| | | 15.00 | 59.20 | 57.20 | 103.5 |
| 3 Serum | 60.62 | 0.00 | 63.30 | | |
| | | 60.00 | 127.50 | 120.62 | 105.7 |
| | | 30.00 | 95.20 | 90.62 | 105.1 |
| | | 15.00 | 76.30 | 75.62 | 100.9 |

9.6 Linearity

| Sample | Dilution | Measured Conc. (nmol/L) | Expected Conc (nmol/L) | Recovery (%) |
|--------|----------|-------------------------|------------------------|--------------|
| 1 | None | 157.00 | 157.00 | |
| | 1:2 | 79.50 | 78.50 | 101.3 |
| | 1:4 | 37.80 | 39.25 | 96.3 |
| | 1:8 | 20.10 | 19.63 | 102.4 |
| | 1:16 | 10.20 | 9.81 | 103.9 |
| 2 | None | 55.10 | 55.10 | |
| | 1:2 | 28.30 | 27.55 | 102.7 |
| | 1:4 | 13.20 | 13.78 | 95.8 |
| | 1:8 | 6.57 | 6.89 | 95.4 |
| 3 | None | 27.80 | 27.80 | |
| | 1:2 | 13.80 | 13.90 | 99.3 |
| | 1:4 | 6.90 | 6.95 | 99.3 |
| | 1:8 | 3.30 | 3.48 | 95.0 |

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.125 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Corticosterone in a sample.

10.3 High-Dose-Hook Effekt

No hook effect was observed in this test.

11 LEGAL ASPECTS**11.1 Reliability of Results**

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IBL International.

11.2 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutical consequences.

11.3 Liability












Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES / LITERATURE

1. Hupe, J.M., et al, *Nature*, 1998, 394, 784-787.
2. Kitaysky A.S., et al, *J. Comp. Physiol*, 2001, 171, 701-709.
3. Thellin O, Noel G, Khuana S, Ogle CK and Horseman N, *Shock*, 2001, 16(5), 393-397.
4. Krame, K.M., Sothern R.B., *Chronobiol. Int.*, 2001, 18(6), 933-945.
5. Vazquez-Palacios G, et al, *Pharmacol. Biochem Behavior*, 2001, 70(2-3), 305-310.

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

| | |
|--|--|
|  | Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.: |
|  | 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: |
|  | Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα |
|  | Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο |
|  | 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. |
|  | Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης. |
|  | 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: / Armazemar a: / Conservare a: / Αποθήκευση στους: |
|  | Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbicante: / Παραγωγός: |
|  | Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή! |
| <p>Symbols of the kit components see MATERIALS SUPPLIED.</p> <p>Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.</p> <p>Voir MATERIEL FOURNI pour les symbôles des composants du kit.</p> <p>Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.</p> <p>Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.</p> <p>Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.</p> <p>Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p> | |

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.

| | | | |
|---|---|--|----------|
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