DRG® Carbonyl Protein (EIA-5141)

RUO

As of 25 Feb. 2010 rm (Vers. 1.0)

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

This ELISA Kit is intended for the determination of protein carbonyls in biological samples such as EDTA-plasma, bronchoalveolar lavage fluid and cerebrospinal fluid, cell extracts and other soluble protein samples. For research use only.

2 INTRODUCTION

Reactive oxygen species (ROS) can oxidize proteins, lipids, and DNA, causing damage of their structure and function as well as cell injury. Proteins are oxidized by free radicals, whereby the constituent amino acids are variously modified or degraded. The modifications result in new functional groups such as carbonyl or hydroxyl groups, which may lead to protein fragmentation, formation of protein-protein cross-linkages, disruption of the tertiary structure and loss of functional activity. In addition, ROS are directly associated with diseases like atherosclerosis, rheumatoid arthritis, Alzheimer's and Parkinson's disease as well as ageing and cancerogenesis.

Protein carbonyls are formed by a variety of oxidative mechanisms and are sensitive indices of oxidative injury. The quantity of protein carbonyls in a protein sample can be determined by derivatizing with dinitrophenylhydrazine (DNPH) and measuring the bound anti-DNPH antibodies. The ELISA method enables carbonyls to be measured quantitatively with microgram quantities of protein.

Indication

- Atherosclerosis
- Alzheimer's disease
- Parkinson's disease
- Rheumatoid arthritis
- Uremia
- Diabetes
- Ageing
- Cancerogenesis

3 PRINCIPLE OF THE TEST

Samples containing protein are reacted with DNPH; then the non-protein constituents and unconjugated DNPH are separated by ultracentrifugation. The proteins are adsorbed to an ELISA plate and incubated with anti-DNPH antibody followed by antibody-linked horseradish peroxidase. Absorbances are related to a standard curve prepared with oxidized serum albumin.

The carbonyl protein content is calculated from the estimated carbonyl concentration and the total protein content of the sample. **For this reason, a parallel determination of the protein content is required**.

4 MATERIAL SUPPLIED

Content	Kit Components	Quantity
PLATE	One holder with strips	12 x 8 wells
WASHBUF	Wash buffer concentrate (10 fold)	1 x 100 ml
STD	Standard stock solution	1 x 50 µl
CTRL	Control, positive	1 x 50 µl
CONJ	Conjugate, peroxidase-labeled	1 x 22 ml
AB	1. Antibody	1 x 240 µl
ABBUF	Antibody dilution buffer	1 x 30 ml
DER	Derivatization reagent	1 x 9 ml
ASYBUF	Assay buffer	2 x 100 ml
SUB	TMB substrate	2 x 15 ml
STOP	Stop solution	1 x 15 ml

5 MATERIAL REQUIRED BUT NOT SUPPLIED

Bidistilled water (aqua bidest.)
Precision pipettors and disposable tips to deliver 0.5 - 1000 µl
Foil to cover the microtiter plate
A multi-channel dispenser or repeating dispenser for washing
Centrifuge capable of 11000 x g
Vortex-Mixer
Standard laboratory **reaction vessels (cups) made of polypropylene**
Centrifugal filtration concentrators can be ordered from DRG (Cat. No K 7822ZR)
Protein quantification test (Cat. No K 7822BCA)
Microtiter plate reader at 450 nm (reference wave length 620 or 690 nm)

6 PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. The kit can be used up to 4 times within the expiry date stated on the label.
- The **WASHBUF** (Wash buffer concentrate) should be diluted with aqua bidest. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C using a water bath before dilution of the buffer solutions. The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label. **Diluted buffer solution** can be stored in a closed flask at **2-8°C for one month**.

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- The **DER** (Derivatization reagent) is prepared as a saturated solution. Crystals can occur due to the high salt concentration. The **DER** (Derivatization reagent) is used as such, without removing the crystals.
- The **AB** (1. Antibody) must be diluted **1:100 in ABBUF** (Antibody dilution buffer): e.g. Preparation of reagents for 1 plate:
  220 µl AB (1. Antibody) + 22 ml ABBUF ((Antibody dilution buffer))
  Diluted AB-solution can be stored **for 2 days at 2-8°C** in a closed flask**.**
- All other test reagents can be stored at 2-8° C and are stable until the expiry date (see label of test package).

7 PRECAUTIONS

Stop as well as derivatization solution is composed of strong acid. Even diluted, they still must be handled with care. They can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.

Reagents should not be used beyond the expiration date shown on kit label.

8 SAMPLE AND TEST PREPARATION

Plasma, bronchoalveolar lavage fluid and cerebrospinal fluid, cell extracts and other soluble protein samples are suited for this test system.

Samples should be sent cooled; they are stable for 24 h at room temperature.

9 ASSAY PROCEDURE

9.1 Procedural notes

The carbonyl protein content is calculated from the estimated carbonyl concentration and the total protein content of the sample. **For this reason, a parallel determination of the protein content is required**.

Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. DRG can therefore not be held reliable for any damage resulting from this.

The assay should always be performed according to the enclosed manual.

9.2 Sample preparation and test procedure

Derivatization

1. Bring all reagents and samples to room temperature (18-26°C)
2. Label the centrifugal filtration concentrators for **STD** (standard), **CTRL** (control), **ASYBUF** (blank) and **SAMPLE** (samples) and place them in the collecting vials
3. Add in each centrifugal filtration concentrator **80 µl** of **DER** (derivatization reagent)
4. Add **4 µl** of each **STD** (standard), **CTRL** (control), **ASYBUF** (blank) and **SAMPLE** (sample) in the corresponding centrifugal filtration concentrator containing the derivatization reagent. Mix by repeated pipetting of the mixture up and down and close the centrifugal filtration concentrator
5. Allow the derivatization to proceed for **45 min** at room temperature

6. Centrifuge all centrifugal filtration concentrators at **11000 x g** for **15 min**
7. **Add 60 µl of ASYBUF** (assay buffer) in all centrifugal filtration concentrators
8. **Repeat step 6 and 7 four times**

Dilution I

1:4 Dilution

- 180 µl ASYBUF (Assay buffer) + 60 µl **Sample** supernatant after derivatization
- 180 µl ASYBUF (Assay buffer) + 60 µl **Control** supernatant after derivatization
- 180 µl ASYBUF (Assay buffer) + 60 µl **Blank** supernatant after derivatization (S6)
- 180 µl ASYBUF (Assay buffer) + 60 µl **Standard** supernatant after derivatization (S1); prepare a dilution series

Standard dilution series

S2= 100 µL S1 + 100 µL ASYBUF (Assay buffer)
S3= 100 µL S2 + 100 µL ASYBUF (Assay buffer)
S4= 100 µL S3 + 100 µL ASYBUF (Assay buffer)
S5= 100 µL S4 + 100 µL ASYBUF (Assay buffer)

Dilution II

1:20 Dilution

- 40 µL Dilution I + 760 µl ASYBUF (Assay buffer)

This dilution is used for protein determination of standard 1 (S1), control and the respective samples. We recommend incubating the protein determination test (BCA-Test) at 37°C for 3 hours.

Dilution III

1:100 Dilution

- 10 µL Dilution II + 990 µl ASYBUF (Assaypuffer)

This dilution is used for the ELISA test.

9.3 Test procedure ELISA

1. Take as many microtiter strips (PLATE) as needed from kit. Store unused strips in the closed original package bag at 2-8°C. Strips are stable until the expiry date stated on the label
2. For the analysis in duplicate, pipette **2 x 200 µl** of **STD** (standards), **CTRL** (control), **BLANK** (blank) and **SAMPLE** (samples) **from dilution III** into the respective well of the microtiter plate
3. Cover plate tightly and incubate for **3 hours at 37°C or over night at 2-8°C**

4. Aspirate the contents of each well. Wash **5 times** by dispensing **250 µl** of **diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
5. Add **200 µl** of **diluted AB** (anti-DNPH-antibody) into each well
6. Cover the plate tightly and incubate for **20 min at room temperature** (18-26°C). Important: Do not shake!
7. Aspirate the contents of each well. Wash **5 times** by dispensing **250 µl** of **diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
8. Add **200 µl** of **CONJ** (conjugate, goat-anti-rabbit-peroxidase-labeled) into each well
9. Cover the plate tightly and incubate for **20 min at room temperature** (18-26°C). Important: Do not shake!
10. Aspirate the contents of each well. Wash **5 times** by dispensing **250 µl** of **diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
11. Add **200 µl** of **SUB** (TMB substrate) into each well
12. Incubate for **15-20 min at room temperature** in the dark*
13. Add **50 µl** of **STOP** (stop solution) into each well, mix thoroughly
14. Determine absorption **immediately** with an ELISA reader **at 450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference

*The intensity of the color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.

10 EVALUATION OF RESULTS

A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from standard. The concentration of patient samples is determined directly from the linear standard curve.
A 4-parameter curve fitting equation is recommended for evaluation of the results.

The protein carbonyl content is calculated according to the following formula:

$$CP_{Sample}\ [pmol/mg]\ standardized = \frac{CP_{Sample}\ [pmol/mg] \times Proteins_{Standard}\ [mg/ml]}{Proteins_{Sample}\ [mg/ml]}$$

CP_{Sample} : Carbonyl protein content of the sample in pmol/mg, estimated from the standard curve in the assay

$Proteins_{Standard}$: Protein content of dilution II of the highest standard (S1), estimated with the BCA-Test in mg/ml

$Proteins_{Sample}$: Protein content of the sample dilution II, estimated with the BCA-Test in mg/ml

10.1 Expected values

Normal range

EDTA-plasma 75 – 200 pmol/mg

11 PERFORMANCE CHARACTERISTICS

11.1 Precision and reproducibility

Intra-Assay (n=4)		
Probe	Carbonyl proteins [pmol/mg]	Standard Deviation (SD) [%]
1	70	9.86
2	140	8.40
3	830	5.80
4	1140	8.40

Inter-Assay (n=4)		
Probe	Carbonyl proteins [pmol/mg	Standard Deviation (SD) [%]

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1	60	7.37
2	170	9.72
3	730	7.19
4	1130	6,36

12 REFERENCES

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13 GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for research use only.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Do not interchange different lot numbers of any kit component within the same assay.
- Guidelines for medical laboratories should be observed.
- The assay should always be performed according the enclosed manual.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. DRG can therefore not be held responsible for any damage resulting from wrong use.

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
Σ		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..