LRG
(LEUCINE-RICH ALPHA-2-GLYCOPROTEIN)
ELISA Assay Kit

(EN) ELISA FOR THE QUANTITATIVE DETERMINATION OF HUMAN LRG
IN SERUM, EDTA PLASMA, HEPARIN PLASMA, AND CITRATE PLASMA
Cat. No. BI-LRG. 12 x 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

For the measurement of LRG in cell culture supernatants or urine please visit our website

www.bmgrp.com

Rev.no. 200827

This kit was developed and manufactured by:
Biomedica Medizinprodukte GmbH, A-1210 Wien, Divischgasse 4
Tel. +43/1/29107 45, Fax +43/1/29107 6389, e-mail info@bmgrp.com

BIOMEDICA

CONTENT

Detailed information on the LRG ELISA, e.g. assay validation data, sample matrix comparisons, and stability data is available on our website. .

www.bmgrp.com

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

1) INTRODUCTION

LRG (leucine-rich alpha-2-glycoprotein) is a glycoprotein with a molecular mass of 38.2 kDa. It is encoded by the human gene LRG-1 which is mapped on chromosome 19 at the cytogenetic band 19p13.3. The protein LRG (or also named LRG1) runs at approximately 50 kDa under reducing conditions, as it contains a carbohydrate content of 23% (1). LRG is the founding member of the family of leucine-rich repeat proteins (2). The mature protein consists of 312 amino acids, from Val36 to Gln347, with a leucine content of 66 amino acids. LRG is folded to eight leucine-rich repeat (LRR) domains of 22 amino acid length, and a C-terminal LRRCT domain with 49 amino acid length (3). Human LRG shows 62.5% sequence identity with mouse LRG, and 60.7% with rat LRG.

LRG binds to the TGFβ accessory receptor endoglin, and in the presence of TGFβ1 this leads to the induction of the TβRII-ALK1-Smad1/5/8 signaling pathway (4). TGFβ1 therefore promotes binding of LRG to the proangiogenic ALK1 but inhibits the interaction with angiostatic ALK5. Induced signaling leads to endothelial cell proliferation and blood vessel outgrowth (4).

Like many other family members of the leucine-rich repeat (LRR) family, LRG has multiple binding partners. LRG directly interacts with the mitochondrial electron transfer protein cytochrome c (5), whereas the physiological relevance of this interaction is not yet known. LRG further binds to TGFβ1, the most frequently expressed TGFβ isoform.

The tissue distribution of LRG varies, with high-level expression in the liver, lower expression in the heart, and minimal expression in spleen and lung (3). LRG is expressed during hematopoiesis. It plays a role in the innate immune system as it is upregulated during neutrophil differentiation; LRG is packed into peroxidase-negative granules of human neutrophils and then secreted upon activation to modulate the microenvironment (6, 7). Differential expression of LRG is further associated with certain carcinomas, neurodegenerative disease, aging or autoimmune disease. In addition, studies have demonstrated an association between cardiac remodeling (hypertrophy, fibrosis, abnormal vasculature, heart failure) and reduced expression of LRG (8, 9).

LRG is involved in cell proliferation and immune response, in cell migration, neovascularization and apoptosis (4, 10, 11). It is a proangiogenic factor which is involved in the regulation of the TGFβ signaling pathway. Up-regulation of LRG is described in response to acute phase response in hepatocytes (12).

LRG is potentially a biomarker for a variety of diseases e.g. as inflammatory biomarker for autoimmune diseases such as rheumatoid arthritis and inflammatory bowel disease (13, 14). Numerous groups have shown that LRG is increased in various immune-related diseases such as psoriasis (15), juvenile idiopathic arthritis (16), Kawasaki disease (17), appendicitis (18), and cancers (19-23), indicating that LRG elevation is not only limited to autoimmune diseases. In addition, LRG may serve as a biomarker for several other disease conditions such as heart failure (24), and diabetes-related complications (25, 26). Plasma Leucine-Rich α-2-Glycoprotein has also been demonstrated to predict cardiovascular disease risk in end-stage renal disease (27). Leucine-rich α-2-glycoprotein is highly expressed in the brain and it is possible to distinguish idiopathic normal pressure hydrocephalus (iNPH) from other neurodegenerative diseases such as Alzheimer disease by measuring LRG in cerebrospinal fluid (28, 29).

<u>Areas of interest:</u>

- Inflammation (Bowel disease, Crohn's disease)
- Autoimmune disease (rheumatoid arthritis, psoriasis)
- Cardiovascular disease (heart failure, ventricular dysfunction, arterial stiffness)
- Kidney disease – diabetic kidney disease
- Cancer
- Appendicitis
- Neurodegenerative diseases

2) CONTENTS OF THE KIT

CONTENTS	KIT COMPONENTS	QUANTITY
PLATE	Sheep polyclonal LRG antibody pre-coated microtiter strips in stripholder packed in aluminium bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer concentrate, 20x, natural cap	1 x 50 ml
ASYBUF	Assay buffer, natural cap with red spot, ready to use	1 x 115 ml
STD	Standards (0, 2, 4, 8, 16, 32, 64 ng/ml), recombinant human LRG, white caps, lyophilised	7 vials
CTRL	Controls A and B, yellow caps, lyophilized, exact concentrations see labels	2 vials

CONJ	Conjugate, (sheep polyclonal anti-human LRG-HRPO), amber cap, ready to use	1 x 13 ml
SUB	Substrate (TMB solution), blue cap, ready to use	1 x 13 ml
STOP	Stop solution, white cap, ready to use	1 x 7 ml

3) ADDITIONAL MATERIAL IN THE KIT

- 2 self-adhesive plastic films
- Quality control protocol
- Protocol sheet
- Instruction for use

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5 µl, 20 µl, 50 µl, 100 µl, 500 µl, 1000 µl and disposable tips
- Distilled or deionised water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Refrigerator with 4°C (2-8°C)
- Microplate reader capable of measuring absorbance at 450 nm (with correction wavelength at 630 nm)
- EP-tubes
- Graph paper or software for calculation of results

5) REAGENTS AND SAMPLE PREPARATION

All reagents as supplied in the kit are stable at 4°C (2-8°C) until the expiry date stated on the label of each reagent.

Sample preparation/dilution:

Collect venous blood samples by using standardized blood collection tubes. Perform plasma or serum separation by centrifugation according to supplier's instructions of the blood collection devices. Assay the acquired samples immediately or aliquot and store at -25°C or lower. Lipemic or haemolyzed samples may give erroneous results. Samples are stable for up to 5 freeze-thaw cycles. Samples should be mixed well before assaying. We recommend duplicates for all values.

Before assaying: Samples must be diluted 1:4000 with assay buffer (ASYBUF) in 2 steps e.g.:
Transfer 5 µl sample to 995 µl ASYBUF (yielding a 1:200 dilution) in an Eppendorf tube. Mix gently.
Next, transfer 20 µl of the 1:200 diluted sample to 380 µl ASYBUF in an Eppendorf tube, resulting in a final sample dilution of 1:4000. Mix gently before testing.

Samples with values above STD7 (64 ng/ml) can be diluted with ASYBUF (Assay buffer) using higher dilution factor than 1:4000. Dilution factor needs to be considered when calculating the final concentration of the sample.

For further information on sample stability please visit our website www.bmgrp.com (see Validation Data) or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

Reagent preparation/handling:

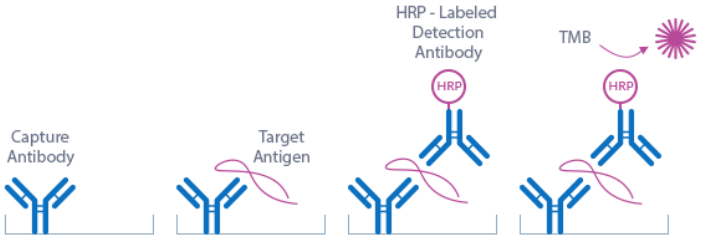
WASHBUF (wash buffer): Dilute the concentrate 1:20, e.g. 50 ml WASHBUF + 950 ml distilled or deionized water. Crystals in the buffer concentrate will dissolve at room temperature. The undiluted WASHBUF is stable at 4°C (2-8°C) until the expiry date stated on label. The diluted WASHBUF is stable up to one month at 4°C (2-8°C). Only use diluted WASHBUF when performing the assay.

STD (standards) + CTRL (controls): Pipette 500 µl of distilled or deionized water into each vial. Leave at room temperature (18-26°C) for 15 min. Vortex. Reconstituted STD and CTRL can be stored at -25°C or lower until expiry date stated on the label. STDs and CTRLs are stable for 3 freeze-thaw cycles.

6) PRINCIPLE OF THE ASSAY

The LRG (leucine-rich alpha-2-glycoprotein) ELISA is a sandwich enzyme immunoassay for the quantitative determination of LRG in human serum and plasma samples. Samples must be pre-diluted 1:4000 prior to assaying, see chapter *5) Reagent and Sample Preparation*. Standards and controls are used undiluted.

In a first step, standards, controls and pre-diluted samples are pipetted into the wells of the microtiter strips, which are pre-coated with polyclonal sheep anti-human LRG antibody. LRG present in the standard/control/sample binds to the pre-coated antibody in the well. All non-specific unbound material is removed in a washing step and the detection antibody (CONJ, polyclonal sheep anti-LRG-HRPO) is pipetted into the wells. After another washing step, the substrate (TMB, tetramethylbenzidine) is added. The enzyme-catalyzed color change of the substrate is directly proportional to the amount of LRG present in the sample. This color change is detectable with a standard microplate reader. A dose response curve of the absorbance (optical density, OD at 450 nm) using the values obtained from the standards versus the standard concentration is generated.
The concentration of LRG in the sample is determined from the dose response curve. **This sample concentration must be multiplied by the dilution factor used for sample preparation to obtain final sample concentration** (e.g. if dilution factor is 1:4000 the concentration obtained from the response curve must be multiplied by 4000).



7) ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-26°C) before use in the assay.
Mark position for STD/CTRL/SAMPLE (standard/control/sample) on the protocol sheet.
Take microtiter strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label.

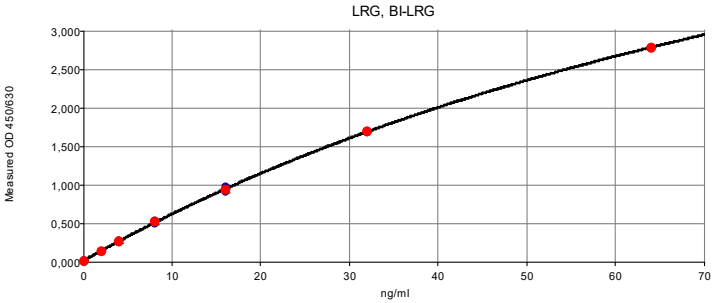
1. Pipette 100 µl STD/CTRL/pre-diluted* SAMPLE in duplicates into respective wells. **for sample pre-dilution 1:4000 with ASYBUF see chapter 5) reagents and sample preparation*
2. **Cover tightly and incubate for 2 hours at room temperature (18-26°C).**
3. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
4. Add 100 µl CONJ (Conjugate, amber cap) into each well, swirl gently.
5. **Cover tightly and incubate for 1 hour at room temperature (18-26°C).**
6. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
7. Add 100 µl SUB (Substrate, blue cap) into each well, swirl gently.
8. **Incubate for 30 min at room temperature (18-26°C) in the dark.**
9. Add 50 µl STOP (Stop solution, white cap) into each well, swirl gently.
10. Measure absorbance immediately at 450 nm with reference 630 nm, if available

8) CALCULATION OF RESULTS

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (correction wavelength 630 nm). Construct the standard curve from the OD values of the STD. Use commercially available software or graph paper. Obtain sample concentration from this standard curve. The assay was evaluated with 4PL algorithm. Different curve fitting methods need to be evaluated by the user.

Respective dilution factors must be considered when calculating the final concentration of the sample (e.g. if dilution factor is 1:4000 the concentration obtained from the response curve must be multiplied by 4000 to obtain final sample concentration).

Example of a typical standard curve: LRG (leucine-rich alpha-2-glycoprotein)



The quality control protocol supplied with the kit shows the results of the final release QC for each kit lot. Data for optical density obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as an OD of 1.50 or more is obtained for the standard with the highest concentration and the values of the CTRLs are in range (target ranges see labels).

9) ASSAY CHARACTERISTICS

Method	Sandwich ELISA, HRP/TMB, 12x8-well detachable strips			
Sample type(s)	Serum, EDTA plasma, citrate plasma, heparin plasma (urine and cell culture protocol available)			
Sample volume	100 µl pre-diluted sample / well (5 µl sample)			
Assay time	2 h / 1 h / 30 min			
Sensitivity	LOD: 0.26 ng/ml; LLOQ: 0.5 ng/ml			
Standard range	0 – 64 ng/ml (0 / 2 / 4 / 8 / 16 / 32 / 64)			
Conversion factor	1 ng/ml = 0.0262 nmol/l; MW: 38.178 kDa			
Precision		**n**	**CV (%)**	
	Within-run	3	≤3	
	In-between-run		*in validation*	
Accuracy (Spike/Recovery of recombinant LRG)		**n**	**Average recovery (%)**	
			+6.4 ng/ml	**+32 ng/ml**
	Serum	5	86	96
	EDTA plasma	5	85	89
	Heparin plasma	5	90	91
	Citrate plasma	2	96	100

		n	Average recovery of expected dilution (%)	
			1+1	1+3
Dilution linearity of endogenous human LRG	**Serum**	5	116	117
	EDTA plasma	7	107	101
	Heparin plasma	1	115	113
	Citrate plasma	1	118	111
Specificity	Endogenous and recombinant human LRG (leucine rich alpha 2 glycoprotein).			
Use	Research use only.			
Values of apparently healthy donors		**n**	**Median LRG (µg/ml)***	
	Serum	18	27.5	
	EDTA plasma	22	27.9	
	Heparin plasma	20	23.8	
	Citrate plasma	22	31.1	

**dilution factor of 1:4000 considered, expressed in µg/ml for better readability*

For further information on assay performance characteristics, matrix comparisons and stability data please visit our website www.bmgrp.com (see Validation Data) or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

10) PRECISION

Within-run (intra-assay): 2 samples of known concentrations were tested 3 times within 1 kit lot by 1 operator.
In-between-run (inter-assay): currently in validation

Within-run (n=3)	Sample 1	Sample 2		Within-run (n=x)	Sample 1	Sample 2
Mean (ng/ml)	3.9	31.7		Mean (ng/ml)		
SD (ng/ml)	0.1	0.8		SD (ng/ml)		
CV (%)	2	3		CV (%)		

11) TECHNICAL HINTS

- Do not mix or substitute reagents with those from other lots or sources.
- Do not mix stoppers and caps from different reagents or use reagents between lots.
- Do not use reagents beyond expiration date.
- Protect reagents from direct sunlight.
- Substrate solution should remain colorless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.

12) PRECAUTIONS

All test components of human source were tested against HIV-Ab, HCV-Ab and HBsAg and were found negative. Nevertheless, they should be handled and disposed as if they were infectious.
All liquid reagents contain ≤0.1% Proclin 950 as preservative. Proclin 950 is not toxic in concentrations used in this kit. It may cause allergic skin reactions – avoid contact with skin or eyes.

- Do not pipette by mouth.
- Do not eat, drink, smoke, or apply cosmetics where reagents are used.
- Wear gloves, glasses, and lab coat while performing this assay.

- Sulfuric acid is irritating to the eyes and skin. Avoid contact with skin and mucous. Irritations are possible. Flush with water if contact occurs!

13) LITERATURE

1. Isolation and characterization of an unknown, leucine-rich 3.1-S-alpha2-glycoprotein from human serum. Haupt H et al., Hoppe Seylers Z Physiol Chem, 1977; 358 (6):639-46.
2. Periodicity of leucine and tandem repetition of a 24-amino acid segment in the primary structure of leucine-rich alpha 2-glycoprotein of human serum. Takahashi N et al., Proc Natl Acad Sci USA, 1985; 82(7): 1906-10.
3. Molecular characterization and expression analysis of leucine-rich alpha2-glycoprotein, a novel marker of granulocytic differentiation. O'Donnell LC et al., J Leukoc Biol, 2002; 72(3): 478-85.
4. LRG1 promotes angiogenesis by modulating endothelial TGF-β signalling. Wang X et al., Nature, 2013; 499(7458): 306-11.
5. Serum leucine-rich alpha-2-glycoprotein-1 binds cytochrome c and inhibits antibody detection of this apoptotic marker in enzyme-linked immunosorbent assay. Cummings C et al., Apoptosis, 2006; 11(7): 1121-9.
6. LRG-accelerated differentiation defines unique G-CSFR signaling pathways downstream of PU.1 and C/EBPepsilon that modulate neutrophil activation. Ai J et al., J Leukoc Biol, 2008; 83(5): 1277-85.
7. Leucine Rich α-2 Glycoprotein: A Novel Neutrophil Granule Protein and Modulator of Myelopoiesis. Druhan LJ et al., PLoS One, 2017; 12; 12(1): e0170261.
8. Elevation of a novel angiogenic factor, leucine-rich-α2-glycoprotein (LRG1), is associated with arterial stiffness, endothelial dysfunction, and peripheral arterial disease in patients with type 2 diabetes. Pek SL et al., J Clin Endocrinol Metab, 2015; 100(4): 1586-93.
9. The role of TGFβ1 and LRG1 in cardiac remodelling and heart failure. Song W et al., Biophys Rev, 2015; 7(1):91-104.
10. LRG1 modulates epithelial-mesenchymal transition and angiogenesis in colorectal cancer via HIF-1α activation. Zhang J et al., J Exp Clin Cancer Res, 2016; 35:29.
11. Stable knockdown of LRG1 by RNA interference inhibits growth and promotes apoptosis of glioblastoma cells in vitro and in vivo. Zhong D et al., Tumour Biol, 2015; 36(6):4271-8.
12. Up-regulation of the expression of leucine-rich alpha(2)-glycoprotein in hepatocytes by the mediators of acute-phase response. Shirai R et al., Biochem Biophys Res Commun, 2009; 382(4): 776-9.
13. iTRAQ-based proteomic identification of leucine-rich alpha-2 glycoprotein as a novel inflammatory biomarker in autoimmune diseases. Serada, S. et al., Ann. Rheum 2010; Dis 69, 770-774.
14. Leucine-rich alpha2-glycoprotein as a potential biomarker for joint inflammation during anti-interleukin-6 biologic therapy in rheumatoid arthritis. Fujimoto M et al., Arthritis Rheumatol, 2015; 67: 2056-2060.
15. Leucine-rich alpha-2 glycoprotein is an innovative biomarker for psoriasis. Nakajima H et al., J Dermatol, 2017; Sci 86, 170-174.
16. Leucine-rich alpha2-glycoprotein as the acute-phase reactant to detect systemic juvenile idiopathic arthritis disease activity during anti-interleukin-6 blockade therapy: A case series. Shimizu M et al., Mod Rheumatol, 2017; 27, 833-837.
17. Identification of candidate diagnostic serum biomarkers for Kawasaki disease using proteomic analysis. Kimura Y. et al., Sci Rep, 2017; 7, 43732.
18. A Novel Noninvasive Appendicitis Score with a Urine Biomarker. Yap TL et al., J Pedriatic Surgery, 2019; 54, 1, 91-96.
19. Overexpression of leucine-rich alpha2-glycoprotein-1 is a prognostic marker and enhances tumor migration in gastric cancer. Yamamoto M et al., Cancer Sci, 2017; 108, 2052-2060.
20. LRG-1 Promotes Pancreatic Cancer Growth and Metastasis via Modulation of the EGFR/P38 Signaling. Xie ZB et al., J Exp & Clin Cancer Res, 2019; 38 (1), 75.
21. Epithelial-mesenchymal Transition via transforming growth factor beta in Pancreatic Cancer Is Potentiated by the Inflammatory Glycoprotein Leucine-Rich Alpha-2 Glycoprotein. Otsuru T et al., Cancer Science, 2019; cas.13918.
22. Validation of LRG1 as a Potential Biomarker for Detection of Epithelial Ovarian Cancer by a Blinded Study. Wu JH et al., PloS One, 2015; 10, 3, e0121112.
23. Leucine-rich alpha-2-glycoprotein-1 is up-regulated in colorectal cancer and is a tumor promoter. Zhang Q et al., OncoTargets and therapy, 2018; 11, 2745–52.
24. Proteomic analysis of coronary sinus serum reveals leucine-rich alpha2-glycoprotein as a novel biomarker of ventricular dysfunction and heart failure. Watson CJ et al., Circ Heart Fail, 2011; 4, 188-197.

25. Plasma Leucine-Rich alpha-2-Glycoprotein 1 Predicts Rapid eGFR Decline and Albuminuria Progression in Type 2 Diabetes Mellitus. Liu JJ et al., J Clin Endocrinol Metab, 2017; 102, 3683-3691.
26. LRG1 Promotes Diabetic Kidney Disease Progression by Enhancing TGF-beta-Induced Angiogenesis. Hong Q et al., J Am Soc Nephrol, 2019; 30, 546-562.
27. Plasma Leucine-Rich α-2-Glycoprotein 1 Predicts Cardiovascular Disease Risk in End-Stage Renal Disease. Yang FJ et al., Sci Rep, 2020; 10, 5988.
28. Brain Localization of Leucine-Rich a2-Glycoprotein and Its Role. Nakajima M et al., Hydrocephalus, Acta Neurochirurgica Supplementum, 113: 97-101.
29. Leucine-rich α-2-glycoprotein is a marker for idiopathic normal pressure hydrocephalus. Nakajima M et al., Acta Neurochir (Wien). 2011; 153(6):1339-46.

SYMBOLS

Expiry date / Verfallsdatum / Date de péremption / Data di scadenza /Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / Doba exspirácie / Doba exspirace

Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de utilización / Consulte as instruções de utilização / Raadpleeg de gebruiksaanwijzing / Se brugsanvisningen / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban foglaltakat / Postupujte podľa pokynov na použitie / Postupujte dle návodu k použití

Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže

Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno

Catalogue Number / Bestellnummer / Numéro de référence / Numero di riferimento / Número de referencia / Número de referência / Referentienummer / Referencenummer / Katalognummer / Numer katalogowy / Katalógusszám / Katalógové číslo / Katalogové číslo

Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk …… között / Skladujte v rozsahu / Skladujte v rozmezí

Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů

LRG ELISA (#BI-LRG)

ASSAY PROTOCOL AND CHECKLIST

PREPARATION OF REAGENTS:

- ❑ Bring all reagents to room temperature (18-26°C).
- ❑ Prepare reagents and samples as instructed.
- ❑ Take microtiter strips out of the aluminium bag and mark positions on the protocol sheet.
- ❑ Bring unused components to the storage temperature mentioned in the package insert.

TEST PROCEDURE:

- ❑ Step 1) Pipette 100 µl STD/CTRL/**pre-diluted** SAMPLE* (standard/control/sample) into respective wells.
- ❑ **Step 2) Cover tightly and incubate for 2 hours at room temperature (18-26°C), in the dark.**
- ❑ Step 3) Aspirate and wash wells with 300 µl WASHBUF (wash buffer, natural cap) five times. Remove remaining buffer by strongly tapping the plate against a paper towel.
- ❑ Step 4) Add 100 µl CONJ (conjugate, amber cap) into each well, swirl gently.
- ❑ **Step 5) Cover tightly and incubate for 1 hour at room temperature (18-26°C), in the dark.**
- ❑ Step 6) Aspirate and wash wells with 300 µl WASHBUF (wash buffer, natural cap) five times. Remove remaining buffer by strongly tapping the plate against a paper towel.
- ❑ Step 7) Add 100 µl SUB (substrate, blue cap) into each well, swirl gently.
- ❑ **Step 8) Incubate for 30 minutes at room temperature (18-26°C), in the dark.**
- ❑ Step 9) Add 50 µl STOP (stop solution, white cap) into each well, swirl gently.
- ❑ Step 10) Measure absorbance immediately at 450 nm with reference 630 nm, if available.

**For sample dilution see chapter 5) Reagent and Sample Preparation*

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

Manufactured by:

BIOMEDICA

Biomedica Medizinprodukte GmbH & Co KG
A-1210 Wien, Divischgasse 4
Tel.+43/1/291 0745 • Fax +43/1/291 0785
www.bmgrp.com • export@bmgrp.com

distributed in the US/Canada by:

EAGLE BIOSCIENCES

EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-411-110
www.EagleBio.com • info@eaglebio.com