DRG® Mouse Interleukin-1ß (96 wells) (EIA-3302 / EIA-3379)

Revised 3 Dec. 2010 rm (Vers. 2.1) **For Research Use Only**

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

Contents and Storage

Storage Store at 2 °C to 8 °C.

Contents

Reagent	96 Test Kit	192 Test Kit
Ms IL-1β Standard, recombinant Ms IL-1β. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
Standard Diluent Buffer. Contains 0.1% sodium azide; 25 mL per bottle.	1 bottle	2 bottles
Incubation Buffer. 12 mL per bottle.	1 bottle	1 bottle
Ms IL-1β High and Low Control, recombinant Ms IL-1β in tissue culture matrix, lyophilized. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume. Once reconstituted, aliquot and store at -20°C or below. Avoid repeated freeze-thaw cycles.	2 vials	2 vials
Antibody Coated Wells. 12 x 8 Well Strips.	1 plate	2 plates
Ms IL-1β Biotin Conjugate (Biotin-labeled anti-Ms IL-1β). Contains 0.1% sodium azide; 6 mL per bottle.	1 bottle	2 bottles
Streptavidin-HRP (100X). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial	2 vials
Streptavidin-HRP Diluent. Contains 3.3 mM thymol; 25 mL per bottle.	1 bottle	1 bottle
Wash Buffer Concentrate *(25X)*. 100 mL per bottle.	1 bottle	1 bottle
Stabilized Chromogen, Tetramethylbenzidine (TMB). 25 mL per bottle.	1 bottle	1 bottle
Stop Solution. 25 mL per bottle.	1 bottle	1 bottle
Plate Covers, adhesive strips.	3	4

Disposal Note
This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Safety
All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Introduction

Purpose

The Mouse Interleukin-1β (Ms IL-1β) ELISA is to be used for the quantitative determination of Ms IL-1β in mouse serum, plasma (EDTA), buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Ms IL-1β.
For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Principle of the Method

The Ms IL-1β kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Ms IL-1β has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Ms IL-1β content, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated monoclonal second antibody.
During the first incubation, the Ms IL-1β antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.
After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Ms IL-1β present in the original specimen.

Background Information

Mouse IL-1β, also known as lymphocyte activating factor, is a 17.5 kilodalton protein which contains 159 amino acids. Primary cellular sources of IL-1β are macrophages and mononcytes, but IL-1β is also produced by lymphocytes, epithelial cells, keratinocytes and mesenchymal cells. There is a 68% amino acid homology between mouse and human forms of IL-1β. IL-1β is best known for mediating the “acute phase” of the inflammatory response. IL-1β mimics shock, is a potent pyrogen, increases the synthesis of normal and pathologic hepatic proteins, induces hypoglycemia, contributes to a negative nitrogen balance, inhibits smooth muscle contraction, and increases the production of several neuropeptides including ACTH, endorphins, vasopressin, and somatostatin (1). IL-1β acts to stabilize mRNA for GM-CSF and induces new transcription of stem cell factors.

Methods

Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
- Calibrated adjustable precision pipettes
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Plastic tubes for diluting solutions
- Absorbent paper towels
- Calibrated beakers and graduated cylinders
- 37 °C Incubator

Procedural Notes

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. **Do not mix or interchange different reagent lots from various kit lots.**
9. Do not use reagents after the kit expiration date.
10. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark.
11. In-house controls or kit controls, if provided, should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. **Never** insert absorbent paper directly into the wells.
13. Because Stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.

Directions for Washing

- **Incomplete washing will adversely affect the test outcome.** All washing must be performed with the Wash Buffer Concentrate (25X) provided.

- Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 mL of diluted Wash Buffer. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- Alternatively, the diluted Wash Buffer may be put into a squirt bottle. If a squirt bottle is used, flood the plate with the diluted Wash Buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- If using an automated washer, follow the washing instructions carefully.

Preparation of Reagents

Preparing Controls

Reconstitute controls in deionized water. Refer to control vial label for instructions. There are two control ranges on the vial:

- The serum/plasma values for the control ranges on the vial label should be used to confirm assay validity for serum/plasma samples.
- The tissue culture values for the control ranges should be used to confirm assay validity for tissue culture supernatant samples. These control ranges were established using a standard curve reconstituted and serially diluted in RPMI supplemented with 10% fetal bovine serum.

Dilution of Standard

One nanogram of Invitrogen recombinant Ms IL-1β equals 2600 units of WHO reference preparation 93/668 (NIBSC, Hertfordshire, UK, EN6 3QG).

Note: Do not use glass tubes for the serial dilution. Use non-glass microcentrifuge or titer tubes.

The standard should be reconstituted to 10,000 pg/mL with **either** Standard Diluent Buffer or Tissue Culture Medium according to the following sample type:

For serum or plasma samples:

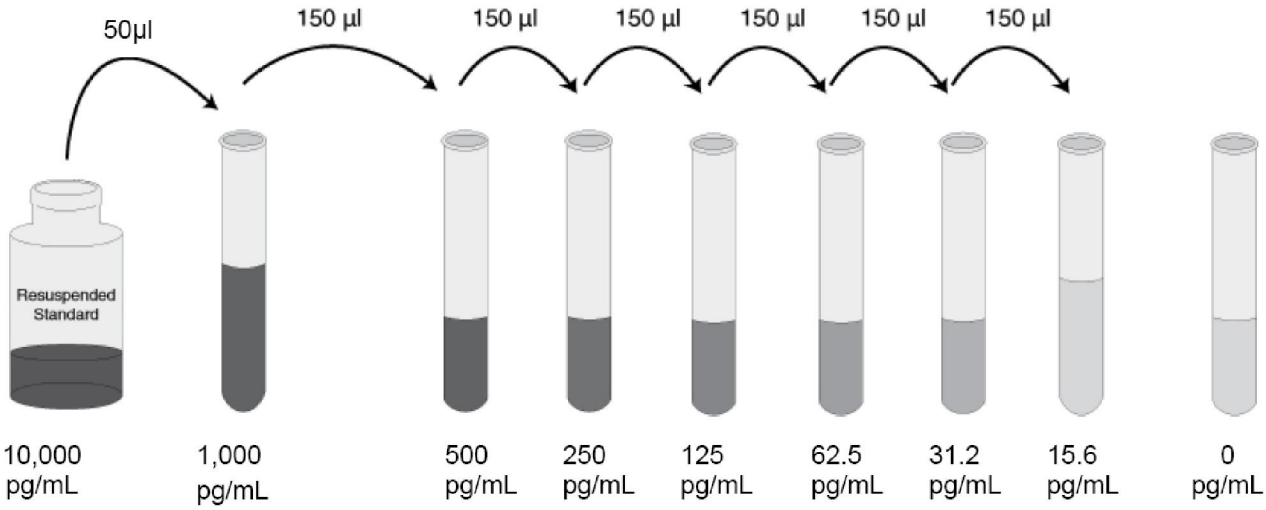
Reconstitute the standard using Standard Diluent Buffer. Serum or plasma samples should be quantified against a standard curve that is reconstituted and serially diluted in Standard Diluent Buffer.

For tissue culture supernatant samples:

Reconstitute the standard using the Tissue Culture Medium used to culture samples. Tissue culture samples should be quantified against a standard curve reconstituted and serially diluted in the Tissue Culture Medium used to culture samples.

1. Add 0.050 mL of the reconstituted standard to a tube containing 0.450 mL Standard Diluent Buffer (for serum and plasma samples) or Tissue Culture Medium (for tissue culture samples).
   Label as 1000 pg/mL Ms IL-1β. Mix.
2. Add 0.150 mL of Standard Diluent Buffer (for serum and plasma samples) or Tissue Culture Medium (for tissue culture samples) to each of 6 tubes labeled 500, 250, 125, 62.5, 31.2, and 15.6 pg/mL Ms IL-1β.
3. Make serial dilutions of the standard as described in the following dilution diagram. Mix thoroughly between steps

Remaining reconstituted standard should be discarded. Return the Standard Diluent Buffer to the refrigerator.



Preparing SAV-HRP

Note: Prepare within 15 minutes of usage. The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution, allow Streptavidin-HRP (100X) to reach room temperature. Gently mix. Pipette Streptavidin-HRP (100X) concentrate slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µL of this 100X concentrated solution with 1 mL of Streptavidin-HRP Diluent for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.
2. Return the unused Streptavidin-HRP (100X) to the refrigerator

# of 8-Well Strips	Volume of Streptavidin-HRP (100X)	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

Dilution of Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the Wash Buffer Concentrate (25X) with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.
2. Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

Assay Procedure

Be sure to read the Procedural Notes section before carrying out the assay.
Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.
Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 50 µL of the Incubation Buffer to all wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 50 µL of the Standard Diluent Buffer (for serum and plasma samples) or Tissue Culture Medium (for tissue culture samples) to the zero standard wells. Well(s) reserved for chromogen blank should be left empty.
4. Add 50 µL of standards, controls, or samples to the appropriate microtiter wells. (See *Preparation of Reagents*.) Tap gently on side of plate to mix.
5. Pipette 50 µL of biotinylated Ms IL-1β Biotin Conjugate solution into each well except the chromogen blank(s). Tap on the side of the plate to thoroughly mix.
6. Cover plate with plate cover and incubate for **1 hour and 30 minutes at 37°C**.
7. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See *Directions for Washing*.
8. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). See *Preparation of Reagents*.
9. Cover plate with the plate cover and incubate for 30 minutes at room temperature.
10. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See *Directions for Washing*.
11. Add 100 µL of Stabilized Chromogen to each well. The liquid in the wells will begin to turn blue.
12. Incubate for **30 minutes at room temperature and in the dark. Note: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the Stop Solution has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
13. Add 100 µL of Stop Solution to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
14. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of Stabilized Chromogen and Stop Solution. Read the plate within 2 hours after adding the Stop Solution.
15. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.

16. Read the concentrations for unknown samples and controls from the standard curve. (Samples producing signals greater than that of the highest standard should be further diluted in Standard Diluent Buffer (serum of plasma) or Tissue Culture Medium (tissue culture samples) and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

Typical Data

(Example)
The following data were obtained for the various standards over the range of 0 to 1,000 pg/mL Ms IL-1β.

Standard Ms IL-1β (pg/mL)	Optical Density (450 nm)
1,000	2.85
500	1.71
250	0.964
125	0.56
62.5	0.34
31.2	0.229
15.6	0.198
0	0.117

Performance Characteristics

Sensitivity

The minimum detectable dose of Ms IL-1β is <7 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Precision

1. Intra-Assay Precision

Samples of known Ms IL-1β concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	188.1	435.0	755.6
SD	8.9	23.8	33.4
%CV	4.7	5.5	4.4

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 30 times in multiple assays to determine precision between assays

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	201.9	400.0	825.8
SD	17.9	29.9	75.4
%CV	8.8	7.4	9.1

SD = Standard Deviation

CV = Coefficient of Variation

Linearity of Dilution

Mouse serum and tissue culture medium containing 10% fetal bovine serum were spiked with Ms IL-1β and serially diluted over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	Serum			Cell Culture		
	Measured (pg/mL)	**Expected (pg/mL)**	**% Expected**	**Measured (pg/mL)**	**Expected (pg/mL)**	**% Expected**
neat	641	-	-	493	-	-
1/2	337	321	105	245	242	99
1/4	183	160	114	130	121	106
1/8	91	80	114	54	60	87
1/16	45	40	113	31	30	101

Recovery

The recovery of Ms IL-1β added to mouse serum averaged 90%
The recovery of Ms IL-1β added to plasma (EDTA) averaged 97%.
The recovery of Ms IL-1β added to tissue culture medium containing 1% fetal bovine serum averaged 101%, while the recovery of Ms IL-1β added to tissue culture medium containing 10% fetal bovine serum averaged 105%.

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Ms IL-1β kit. The following substances were tested and found to have no cross-reactivity:
human IL-1β, IL-2, IL-4, IL-6, IL-7, IL-10, IFN-γ, TNF-α;
mouse IL-1α IL-2, IL-3, IL-4, IL-6, IL-10, IFN-γ, TNF-α;
rat IL-1α, IFN-γ; swine IL-1β and rat IL-1β showed 0.3% and 12% cross-reactivity, respectively.

High Dose Hook Effect

A sample spiked with Ms IL-1β up to 39 ng/mL gave a response higher than that obtained for the last standard point.

Expected Values

Ten sera and ten plasma (EDTA) samples were evaluated in this assay.
The values for sera ranged from 0 to 18 pg/mL (mean = 6.6 pg/mL).

The values for plasma ranged from 0 to 19 pg/mL (mean = 6.8 pg/mL).

Mouse splenocytes were cultured under the following conditions and the culture supernatants were assayed for released Ms IL-1β.
LPS (25 mg/mL), PHA (5 mg/mL) 4 hr: 17 pg/mL
LPS (25 mg/mL), PHA (5 mg/mL) 24 hr: 41 pg/mL
Con-A (5 mg/mL) 6 hr: 0 pg/mL
PMA (50 ng/mL), Ionophore (250 ng/mL) 12 hr: 61 pg/mL

Limitations of the Procedure

Do not extrapolate the standard curve beyond the top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the top standard point with Standard Diluent Buffer; reanalyze these and multiply results by the appropriate dilution factor.
The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Ms IL-1β in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Troubleshooting Guide

Elevated background

Cause: Insufficient washing and/or draining of wells after washing. Solution containing either biotin or Streptavidin-HRP can elevate the background if residual is left in the well.
Solution: Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain and tap forcefully if necessary to remove residual fluid.

Cause: Contamination of substrate solution with metal ions or oxidizing reagents.
Solution: Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil.

Cause: Contamination of pipette, dispensing reservoir or substrate solution with SAV-HRP conjugate.
Solution: Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen.

Cause: Incubation time is too long or incubation temperature is too high.
Solution: Reduce incubation time and/or temperature.

Elevated sample/standard ODs

Cause: Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly.
Solution: Follow the protocol instructions regarding the dilution of the standard.

Cause: Incorrect dilution of the Streptavidin-HRP Working Solution.
Solution: Warm solution of Streptavidin-HRP (100X) to room temperature, draw up slowly and wipe tip with kim-wipe to remove excess. Dilute ONLY in Streptavidin-HRP Diluent provided.

Cause: Incubation times extended.
Solution: Follow incubation times outlined in protocol.

Cause: Incubations carried out at 37°C when RT is dictated.
Solution: Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.

Poor standard curve

Cause: Improper preparation of standard stock solution.
Solution: Dilute lyophilized standard as directed by the vial label only with the standard diluent buffer or in a diluent that most closely matches the matrix of your sample.

Cause: Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted.
Solution: NEVER substitute any components from another kit.

Cause: Errors in pipetting the standard or subsequent steps.
Solution: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.

Weak/no color develops

Cause: Reagents not at RT (25 ± 2°C) at start of assay.
Solution: Allow ALL reagents to warm to RT prior to commencing assay.

Cause: Incorrect storage of components, e.g., not stored at 2 to 8°C.
Solution: Store all components exactly as directed in protocol and on labels.

Cause: Working Streptavidin-HRP solution made up longer than 15 minutes before use in assay.
Solution: Use the diluted Streptavidin-HRP within 15 minutes of dilution.

Cause: TMB solution lost activity.
Solution 1: The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded.
Solution 2: Avoid contact of the TMB solution with items containing metal ions.

Cause: Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized.
Solution: Please contact Technical Support for advice when using nonvalidated sample types.

Cause: Wells have been scratched with pipette tip or washing tips.
Solution: Use caution when dispensing and aspirating into and out of microwells.

Poor Precision

Cause: Errors in pipetting the standards, samples or subsequent steps.
Solution: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip.

Cause: Repetitive use of tips for several samples or different reagents.
Solution: Use fresh tips for each sample or reagent transfer.

Cause: Wells have been scratched with pipette tip or washing tips.
Solution: Use caution when dispensing and aspirating into and out of microwells.

References / Literature

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Limited Warranty

DRG is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an product or service, please contact our Technical Support Representatives. DRG warrants that all of its products will perform according to the specifications stated on the Certificate of Analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits DRG's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. DRG reserves the right to select the method(s) used to analyze a product unless DRG agrees to a specified method in writing prior to acceptance of the order. DRG makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore DRG makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives. DRG assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose

Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial 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
	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