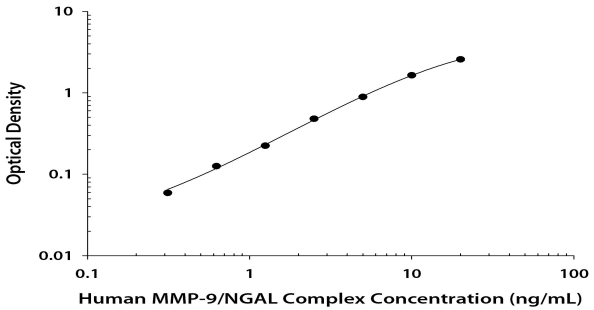
Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human MMP-9/NGAL complex concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data

This standard curve is only for demonstration purposes. A standard curve should be generated for each set of samples assayed.



Specificity

The following factors prepared at 200 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:
Lipocalin-1
Lipocalin-2
MMP-2
TIMP-1

Recombinant human MMP-9 and recombinant human MMP-9/TIMP-1 complex do not cross react in this assay but do interfere at concentrations > 100 ng/mL and 25 ng/mL respectively.

Technical Hints & Limitations

- We recommend the use of R&D Systems® Reagent Diluent Concentrate 2 (Catalog # DY995) to prepare Reagent Diluent and Block Buffer for use in this assay.
- If assaying sample types other than cell culture supernates, each laboratory should develop and validate its own diluent. Do not use buffers with animal serum to reconstitute the detection antibody or to dilute the Streptavidin-HRP B.
- It is important that the Reagent Diluent selected for reconstitution and dilution of the standard reflects the environment of the samples being measured.
- Avoid microbial contamination of reagents and buffers.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is recommended that all standards and samples be assayed in duplicate.
- The use of PBS from tablets may interfere in this assay.

Troubleshooting

Note: *For more detailed troubleshooting, please visit:*
www.rndsystems.com/elisadevelopment

Poor Standard Curve

- Impure BSA used for Block Buffer preparation.
- Improper reconstitution and/or storage of standard.
- Improper dilution of highest standard and standard curve.
- Incomplete washing and/or aspiration of wells.
- Unequal volumes added to wells/pipetting error.
- Incorrect incubation times or temperatures.

Poor Precision

- Unequal volumes added to wells/pipetting error.
- Incomplete washing and/or aspiration of wells.
- Unequal mixing of reagents.

Low or No Color Development

- Inadequate volume of substrate added to wells.
- Incorrect incubation times or temperatures.
- Impure BSA used for Block Buffer preparation.

DuoSet™ ELISA Development System

Human MMP-9/NGAL Complex

Catalog Number: DY8556-05 (5 plates)

Intended Use

For the development of sandwich ELISAs to measure natural and recombinant human MMP-9/NGAL complex. The Reagent Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The Reagent Diluent selected for use can alter the performance of an immunoassay. Reagent Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay.

This kit contains sufficient materials to run ELISAs on 96 well plates, provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

This package insert must be read in its entirety before using this product. Refer to the Certificate of Analysis for component concentrations as they may vary. For research use only. Not for use in diagnostic procedures.

Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL:800 343 7475 612 379 2956 FAX:612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475 FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

Other Materials & Solutions Required

96 well microplates: (R&D Systems®, Catalog # DY990)

Plate Sealers: (R&D Systems, Catalog # DY992)

PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY006)

Wash Buffer: 0.05% Tween®; 20 in PBS, pH 7.2-7.4 (R&D Systems, Catalog # WA126)

Block Buffer: 1% BSA in PBS, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY995).
Quality of BSA is critical (see Technical Hints)

Reagent Diluent: 50 mM Tris, 10 mM $CaCl_2$, 0.15 M NaCl, 0.05% Brij® 35, pH 7.45-7.55, 0.2 µm filtered

Substrate Solution: ELISA TMB Substrate (R&D Systems, Catalog # DY999B or DY999B-250)

ELISA Stop Solution: Methanesulfonic acid (R&D Systems, Catalog # DY994B or DY994B-250)

Also Required:

Normal Goat Serum: (R&D Systems, Catalog # DY005)

Precautions

This analyte is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

The Stop Solution suggested for use with this kit is an acid solution.

The human plasma used for the isolation of MMP-9/NGAL Complex Standard was certified by the supplier to be HIV-1 and HBsAg negative at the time of shipment. Human blood products should always be treated in accordance with universal handling precautions.

Calibration

This DuoSet is calibrated against human MMP-9/NGAL complex purified from stimulated human Neutrophils..

Materials Provided

Store the unopened kit 2-8 °C. Do not use past kit expiration date.

Description	Part #
Human MMP-9/NGAL Complex Capture Antibody	844426
Human MMP-9/NGAL Complex Detection Antibody	844427
Human MMP-9/NGAL Complex Standard	844428
Streptavidin-HRP B	893975

Reagent Preparation

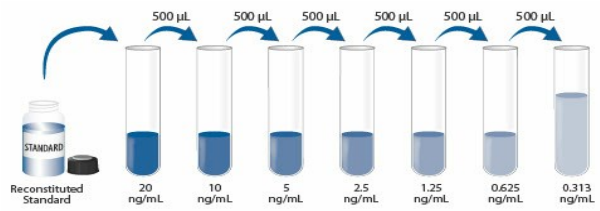
Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Streptavidin-HRP B: 2.0 mL of streptavidin conjugated to horseradish-peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

Human MMP-9/NGAL Complex Capture Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute with 0.5 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the C of A.

Human MMP-9/NGAL Complex Detection Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent with 2 % heat inactivated normal goat serum (NGS) to the working concentration indicated on the C of A.

Human MMP-9/NGAL Complex Standard: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of Deionized Water. A 7 point standard curve using 2-fold serial dilutions in Reagent Diluent is recommended. Prepare 1000 µL of high standard per plate assayed at the concentration indicated on the C of A.



General ELISA Protocol

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of block buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
3. Add 100 µL of the Detection Antibody, diluted in Reagent Diluent with NGS, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
5. Add 100 µL of the working dilution of Streptavidin-HRP B to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.