

**ab119600 –
Human Lipocalin-2
(NGAL) ELISA Kit**
With Wash Buffer (25x)

Instructions for Use

For quantitative detection of Human Lipocalin-2 (NGAL) in cell culture supernatants, serum, plasma (heparin), saliva and urine

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's Human Lipocalin-2 (NGAL) *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Human Lipocalin-2 in cell culture supernatants, serum, plasma (heparin), saliva and urine.

A Lipocalin-2 specific mouse monoclonal antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and incubated. A biotinylated detection polyclonal antibody from goat specific for Lipocalin-2 is then added followed by washing with 1X Wash Buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with 1X Wash Buffer. TMB is then used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the Human Lipocalin-2 amount of sample captured in plate.

Lipocalin-2 (LCN2), also known as NGAL, is a protein associated with neutrophil gelatinase. The LCN2 gene is located at 9q34 and contains 7 exons. The 25-kD LCN2 protein is believed to bind small lipophilic substances such as bacteria-derived lipopolysaccharide (LPS) and formylpeptides and may function as a modulator of inflammation. Lipocalin-2 tightly binds bacterial catecholate-type ferric siderophores through a cyclically permuted, hybrid electrostatic/cation- π interaction and is a potent bacteriostatic agent in iron-limiting conditions. The primary LCN2 transcript is 3,696 nucleotides long, and the processed transcript is 809 nucleotides long. LCN2 expression in adult bone marrow, uterus, prostate, salivary gland, stomach, appendix, colon, trachea, and lung, and in fetal spleen and lung. The standard product used in this kit is recombinant Human Lipocalin-2, consisting of 178 amino acids with the molecular mass of 22KDa.

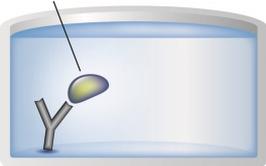
2. ASSAY SUMMARY

Primary Capture Antibody



Prepare all reagents, samples and standards as instructed.

Sample



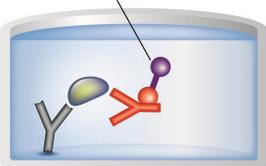
Add standard or sample to each well used. Incubate at room temperature.

Biotinylated Antibody



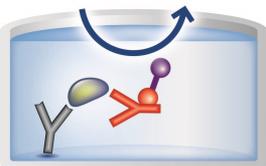
Add prepared biotin antibody to each well. Incubate at room temperature.

Avidin-Biotin-Peroxidase Complex



Add prepared Avidin-Biotin-Peroxidase Complex (ABC). Incubate at room temperature.

Substrate Colored Product



Add TMB to each well. Incubate at room temperature. Add Stop Solution to each well. Read

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles.

Refer to list of materials supplied for storage conditions of individual components.

5. MATERIALS SUPPLIED

| Item | Amount | Storage Condition (Before Preparation) |
|---|-----------|--|
| Anti-Human Lipocalin-2 antibody Microplate (12 x 8 wells) | 96 Wells | -20°C |
| Lyophilized recombinant Human Lipocalin-2 standard | 2 x 10 ng | -20°C |
| Biotinylated anti-Human Lipocalin-2 antibody | 100 µL | -20°C |
| Avidin-Biotin-Peroxidase Complex (ABC) | 100 µL | -20°C |
| Sample Diluent Buffer | 30 mL | -20°C |
| Antibody Diluent Buffer | 12 mL | -20°C |
| ABC Diluent Buffer | 12 mL | -20°C |
| TMB Color Developing Agent | 10 mL | -20°C |
| TMB Stop Solution | 10 mL | -20°C |
| Plate Seal | 4 units | -20°C |
| Wash Buffer (25X) | 20 mL | -20°C |

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Standard microplate reader
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- To determine the appropriate sample dilution to use in this ELISA a pilot experiment using standards and a small number of samples is recommended

GENERAL INFORMATION

- The TMB Color Developing agent is colorless and transparent before use
- Before using the kit, briefly centrifuge the tubes in case any of the contents are trapped in the lid
- It is recommended to assay all standards, controls and samples in duplicate
- Do not let the 96-well plate dry out as this will inactivate active components on plate
- To avoid cross contamination do not reuse tips and tubes
- In order to avoid marginal effects of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before using
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 1X Biotinylated anti-Human Lipocalin-2

Biotinylated anti-Human Lipocalin-2 antibody must be diluted 1:100 with the Antibody Diluent Buffer and mixed thoroughly (i.e. add 1 μL Biotinylated anti-Human Lipocalin-2 antibody to 99 μL Antibody Diluent Buffer.) The total volume required should be; 100 μL /well multiplied by the total number of wells (allow 100 μL - 200 μL extra for pipetting error).

9.2 1X Avidin-Biotin-Peroxidase Complex

Before use, briefly centrifuge the tubes in case any of the contents are trapped in the lid or sticking to the tube walls.

Avidin-Biotin-Peroxidase Complex (ABC) must be diluted 1:100 with ABC Diluent Buffer and mixed thoroughly (i.e. add 1 μL ABC to 99 μL ABC Diluent Buffer.) The total volume required should be; 100 μL /well multiplied by the total number of wells (allow 100 μL - 200 μL extra for pipetting error).

9.3 Wash Buffer (25X)

Prepare 500 mL of working 1X Wash Buffer by diluting 20 ml of the supplied Wash Buffer (25X) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Reconstitution of the Human Lipocalin-2 standard should be prepared no more than 2 hours prior to the experiment. Two tubes of Lipocalin-2 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

- 10.1 Prepare a 10 ng/mL **Standard #1** by reconstituting the Lipocalin-2 standard with addition of 1 mL Sample Diluent Buffer. Hold at room temperature for 10 minutes. This should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.
- 10.2 Label seven tubes with #2 - 8.
- 10.3 Add 300 µL Sample Diluent Buffer into tubes #2 - 8.
- 10.4 Prepare **Standard #2** by transferring 300 µL from Standard #1 to tube #2. Mix thoroughly and gently.
- 10.5 Prepare **Standard #3** by transferring 300 µL from Standard #2 to tube #3. Mix thoroughly and gently.
- 10.6 Prepare **Standard #4** by transferring 300 µL from Standard #3 to tube #4. Mix thoroughly and gently.
- 10.7 Using the table below as a guide, repeat for tubes #5 through #7.
- 10.8 **Standard #8** contains no protein and is the Blank control.

ASSAY PREPARATION

| Standard # | Sample to Dilute | Volume to Dilute (μ L) | Volume of Diluent (μ L) | Starting Conc. (pg/mL) | Final Conc. (pg/mL) |
|------------|------------------|-----------------------------|------------------------------|------------------------|---------------------|
| 1 | Step 10.1 | | | | 10,000 |
| 2 | Standard #1 | 300 | 300 | 10,000 | 5,000 |
| 3 | Standard #2 | 300 | 300 | 5,000 | 2,500 |
| 4 | Standard #3 | 300 | 300 | 2,500 | 1,250 |
| 5 | Standard #4 | 300 | 300 | 1,250 | 625 |
| 6 | Standard #5 | 300 | 300 | 625 | 312 |
| 7 | Standard #5 | 300 | 300 | 312 | 156 |
| 8 | None | - | 300 | - | - |



11. SAMPLE COLLECTION AND STORAGE

Store samples to be assayed within 24 hours at 2 - 8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

11.1 Cell Culture Supernatants

Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

11.2 Serum

Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x *g* for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

11.3 Plasma

Collect plasma using heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.

11.4 Saliva:

Collect saliva using a collection device without any protein binding or filtering capabilities such as a Salivette or aliquot and store samples at -20°C.

11.5 Urine:

Aseptically collect the first urine of the day; micturate directly into a sterile container. Remove particular impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.

SAMPLE PREPARATION

General Sample information:

The user needs to estimate the concentration of the target protein in the sample and select the correct dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

Dilute the samples using the provided Sample Diluent Buffer. The following is a guideline for sample dilution. Several trials may be necessary to determine the optimal dilution factor. The sample must be thoroughly mixed with the Sample Diluent Buffer before assaying.

- High target protein concentration (100 – 1,000 ng/mL). The working dilution is 1:100. i.e. Add 1 μ L sample into 99 μ L Sample Diluent Buffer
- Medium target protein concentration (10 - 100 ng/mL). The working dilution is 1:10. i.e. Add 10 μ L sample into 90 μ L Sample Diluent Buffer
- Low target protein concentration (156 - 10,000 pg/mL). The working dilution is 1:2. i.e. Add 50 μ L sample to 50 μ L Sample Diluent Buffer
- Very Low target protein concentration (\leq 156 pg/mL). No dilution necessary, or the working dilution is 1:2.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

14.1 Prepare all reagents, working standards, and samples as directed in the previous sections

14.2 Add 100 μ L of prepared standards and diluted samples to appropriate wells.

14.3 Seal the plate with a new plate seal and incubate at 37°C for 90 minutes.

14.4 Remove the plate seal, discard contents of each well, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

14.5 Add 100 μ L of 1X Biotinylated anti-Human Lipocalin-2 antibody into each well, seal the plate with a new plate seal and incubate the plate at 37°C for 60 minutes.

14.6 Wash the plate three times with 300 μ L 1X Wash Buffer, and each time let the wash buffer stay in the wells for one minute. Discard the wash buffer and blot the plate onto paper towels or other absorbent material.

Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with each wash. Blot the plate onto paper towels or other absorbent material.

14.7 Add 100 μ L of 1X Avidin-Biotin-Peroxidase Complex working solution into each well, seal the plate with a new plate seal and incubate the plate at 37°C for 30 minutes.

14.8 Wash plate five times with 1X Wash Buffer, and each time let washing buffer stay in the wells for 1 - 2 minutes. Discard the wash buffer and blot the plate onto paper towels or other absorbent material. (See Step 14.6 for plate washing method).

ASSAY PROCEDURE

- 14.9 Add 90 μL of prepared TMB color developing agent into each well, seal the plate with a new plate seal and incubate plate at 37°C in dark for 15 - 25 minutes

Note: The optimal incubation time should be determined by end user. The shades of blue should be seen in the wells with the four most concentrated Human Lipocalin-2 standard solutions; the other wells show no obvious color.

- 14.10 Add 100 μL of prepared TMB Stop Solution into each well. The color changes into yellow immediately.
- 14.11 Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

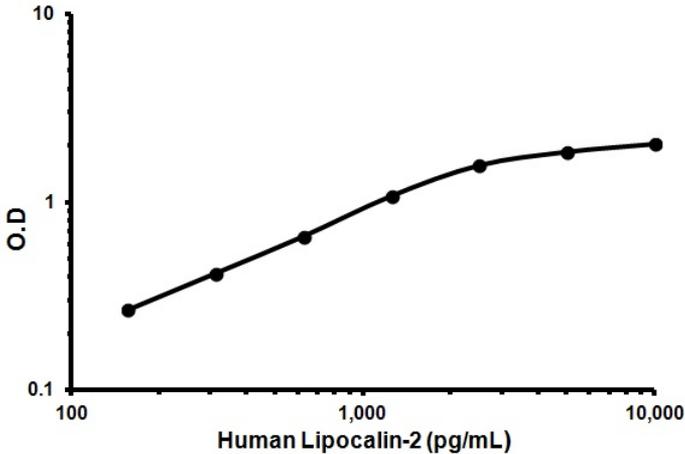
14. CALCULATIONS

For calculation, the relative O.D.450 = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human Lipocalin-2 concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, make sure to account for this in your calculations.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



| Conc. (pg/mL) | O.D. 450nm |
|---------------|------------|
| 0 | 0.075 |
| 156 | 0.267 |
| 312 | 0.418 |
| 625 | 0.657 |
| 1,250 | 1.075 |
| 2,500 | 1.563 |
| 5,000 | 1.842 |
| 10,000 | 2.028 |

16. TYPICAL SAMPLE VALUES

RANGE – 156 – 10,000 pg/mL

SENSITIVITY – < 10 pg/mL

17. ASSAY SPECIFICITY

This kit detects both endogenous and recombinant Human Lipocalin-2.

No detectable cross-reactivity with other relevant proteins.

18. TROUBLESHOOTING

| Problem | Cause | Solution |
|---|---|--|
| Poor standard curve | Inaccurate pipetting | Check pipettes |
| | Improper standards dilution | Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing |
| Low Signal | Incubation times too brief | Ensure sufficient incubation times; change to overnight standard/sample incubation |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| Samples give higher value than the highest standard | Starting sample concentration is too high. | Dilute the specimens and repeat the assay |
| Large CV | Plate is insufficiently washed | Review manual for proper wash technique. If using a plate washer, check all ports for obstructions |
| | Contaminated wash buffer | Prepare fresh wash buffer |
| Low sensitivity | Improper storage of the kit | Store the all components as directed. |

19. NOTES

Technical Support

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For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)

ab119600 – Human Lipocalin-2 (NGAL) ELISA Kit

Without Wash Buffer (25x)

Instructions for Use

For quantitative detection of Human Lipocalin-2 (NGAL) in cell culture supernatants, serum, plasma (heparin), saliva and urine

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1. BACKGROUND

Abcam's Human Lipocalin-2 (NGAL) *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Human Lipocalin-2 in cell culture supernatants, serum, plasma (heparin), saliva and urine.

A Lipocalin-2 specific mouse monoclonal antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and incubated. A biotinylated detection polyclonal antibody from goat specific for Lipocalin-2 is then added followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. TMB is then used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the Human Lipocalin-2 amount of sample captured in plate.

Lipocalin-2 (LCN2), also known as NGAL, is a protein associated with neutrophil gelatinase. The LCN2 gene is located at 9q34 and contains 7 exons. The 25-kD LCN2 protein is believed to bind small lipophilic substances such as bacteria-derived lipopolysaccharide (LPS) and formylpeptides and may function as a modulator of inflammation. Lipocalin-2 tightly binds bacterial catecholate-type ferric siderophores through a cyclically permuted, hybrid electrostatic/cation- π interaction and is a potent bacteriostatic agent in iron-limiting conditions. The primary LCN2 transcript is 3,696 nucleotides long, and the processed transcript is 809 nucleotides long. LCN2 expression in adult bone marrow, uterus, prostate, salivary gland, stomach, appendix, colon, trachea, and lung, and in fetal spleen and lung. The standard product used in this kit is recombinant Human Lipocalin-2, consisting of 178 amino acids with the molecular mass of 22KDa.

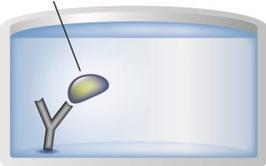
2. ASSAY SUMMARY

Primary Capture Antibody



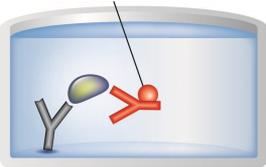
Prepare all reagents, samples and standards as instructed.

Sample



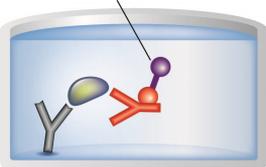
Add standard or sample to each well used. Incubate at room temperature.

Biotinylated Antibody



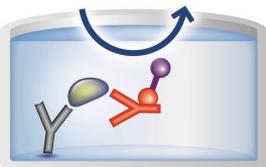
Add prepared biotin antibody to each well. Incubate at room temperature.

Avidin-Biotin-Peroxidase Complex



Add prepared Avidin-Biotin-Peroxidase Complex (ABC). Incubate at room temperature.

Substrate Colored Product



Add TMB to each well. Incubate at room temperature. Add Stop Solution to each well. Read

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4 STORAGE AND STABILITY

Store kit at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles.

Refer to list of materials supplied for storage conditions of individual components.

5. MATERIALS SUPPLIED

| Item | Amount | Storage Condition (Before Preparation) |
|---|-----------|--|
| Anti-Human Lipocalin-2 antibody Microplate (12 x 8 wells) | 96 Wells | -20°C |
| Lyophilized recombinant Human Lipocalin-2 standard | 2 x 10 ng | -20°C |
| Biotinylated anti-Human Lipocalin-2 antibody | 100 µL | -20°C |
| Avidin-Biotin-Peroxidase Complex (ABC) | 100 µL | -20°C |
| Sample Diluent Buffer | 30 mL | -20°C |
| Antibody Diluent Buffer | 12 mL | -20°C |
| ABC Diluent Buffer | 12 mL | -20°C |
| TMB Color Developing Agent | 10 mL | -20°C |
| TMB Stop Solution | 10 mL | -20°C |
| Plate Seal | 4 units | -20°C |

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Standard microplate reader
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Washing buffer, either neutral PBS or TBS (see Section 9 for recipes)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- To determine the appropriate sample dilution to use in this ELISA a pilot experiment using standards and a small number of samples is recommended
- The TMB Color Developing agent is colorless and transparent before use
- Before using the kit, briefly centrifuge the tubes in case any of the contents are trapped in the lid
- It is recommended to assay all standards, controls and samples in duplicate
- Do not let the 96-well plate dry out as this will inactivate active components on plate
- To avoid cross contamination do not reuse tips and tubes
- In order to avoid marginal effects of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before using
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 1X Biotinylated anti-Human Lipocalin-2

Biotinylated anti-Human Lipocalin-2 antibody must be diluted 1:100 with the Antibody Diluent Buffer and mixed thoroughly (i.e. add 1 µL Biotinylated anti-Human Lipocalin-2 antibody to 99 µL Antibody Diluent Buffer.) The total volume required should be; 100 µL/well multiplied by the total number of wells (allow 100 µL - 200 µL extra for pipetting error).

9.2 1X Avidin-Biotin-Peroxidase Complex

Before use, briefly centrifuge the tubes in case any of the contents are trapped in the lid or sticking to the tube walls.

Avidin-Biotin-Peroxidase Complex (ABC) must be diluted 1:100 with ABC Diluent Buffer and mixed thoroughly (i.e. add 1 µL ABC to 99 µL ABC Diluent Buffer.) The total volume required should be; 100 µL/well multiplied by the total number of wells (allow 100 µL - 200 µL extra for pipetting error).

9.3 0.01 M TBS

Add 1.2 g Tris, 8.5 g NaCl; 450 µL of purified acetic acid or 700 µL of concentrated hydrochloric acid to distilled water and adjust pH to 7.2 - 7.6. Finally, adjust the total volume to 1 L with distilled water.

9.4 0.01 M PBS

Add 8.5 g NaCl, 1.4 g Na₂HPO₄ and 0.2 g NaH₂PO₄ to distilled water and adjust pH to 7.2 - 7.6. Finally, adjust the total volume to 1 L with distilled water.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Reconstitution of the Human Lipocalin-2 standard should be prepared no more than 2 hours prior to the experiment. Two tubes of Lipocalin-2 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

- 10.1 Prepare a 10 ng/mL **Standard #1** by reconstituting the Lipocalin-2 standard with addition of 1 mL Sample Diluent Buffer. Hold at room temperature for 10 minutes. This should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.
- 10.2 Label seven tubes with #2 - 8.
- 10.3 Add 300 µL Sample Diluent Buffer into tubes #2 - 8.
- 10.4 Prepare **Standard #2** by transferring 300 µL from Standard #1 to tube #2. Mix thoroughly and gently.
- 10.5 Prepare **Standard #3** by transferring 300 µL from Standard #2 to tube #3. Mix thoroughly and gently.
- 10.6 Prepare **Standard #4** by transferring 300 µL from Standard #3 to tube #4. Mix thoroughly and gently.
- 10.7 Using the table below as a guide, repeat for tubes #5 through #7.
- 10.8 **Standard #8** contains no protein and is the Blank control.

ASSAY PREPARATION

| Standard # | Sample to Dilute | Volume to Dilute (μL) | Volume of Diluent (μL) | Starting Conc. (pg/mL) | Final Conc. (pg/mL) |
|------------|------------------|------------------------------------|-------------------------------------|------------------------|---------------------|
| 1 | Step 10.1 | | | | 10,000 |
| 2 | Standard #1 | 300 | 300 | 10,000 | 5,000 |
| 3 | Standard #2 | 300 | 300 | 5,000 | 2,500 |
| 4 | Standard #3 | 300 | 300 | 2,500 | 1,250 |
| 5 | Standard #4 | 300 | 300 | 1,250 | 625 |
| 6 | Standard #5 | 300 | 300 | 625 | 312 |
| 7 | Standard #5 | 300 | 300 | 312 | 156 |
| 8 | None | - | 300 | - | - |



11. SAMPLE COLLECTION AND STORAGE

Store samples to be assayed within 24 hours at 2 - 8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

11.1 Cell Culture Supernatants

Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

11.2 Serum

Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x *g* for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

11.3 Plasma

Collect plasma using heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.

11.4 Saliva:

Collect saliva using a collection device without any protein binding or filtering capabilities such as a Salivette or aliquot and store samples at -20°C.

11.5 Urine:

Aseptically collect the first urine of the day; micturate directly into a sterile container. Remove particular impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.

SAMPLE PREPARATION

General Sample information:

The user needs to estimate the concentration of the target protein in the sample and select the correct dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

Dilute the samples using the provided Sample Diluent Buffer. The following is a guideline for sample dilution. Several trials may be necessary to determine the optimal dilution factor. The sample must be thoroughly mixed with the Sample Diluent Buffer before assaying.

- High target protein concentration (100 – 1,000 ng/mL). The working dilution is 1:100. i.e. Add 1 μ L sample into 99 μ L Sample Diluent Buffer
- Medium target protein concentration (10 - 100 ng/mL). The working dilution is 1:10. i.e. Add 10 μ L sample into 90 μ L Sample Diluent Buffer
- Low target protein concentration (156 - 10,000 pg/mL). The working dilution is 1:2. i.e. Add 50 μ L sample to 50 μ L Sample Diluent Buffer
- Very Low target protein concentration (\leq 156 pg/mL). No dilution necessary, or the working dilution is 1:2.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
 - a. Prepare all reagents, working standards, and samples as directed in the previous sections
 - b. Add 100 μ L of prepared standards and diluted samples to appropriate wells.
 - c. Seal the plate with a new plate seal and incubate at 37°C for 90 minutes.
 - d. Remove the plate seal, discard contents of each well, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
 - e. Add 100 μ L of 1X Biotinylated anti-Human Lipocalin-2 antibody into each well, seal the plate with a new plate seal and incubate the plate at 37°C for 60 minutes.
 - f. Wash the plate three times with 300 μ L 0.01 M TBS or 0.01 M PBS, and each time let the washing buffer stay in the wells for one minute. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with each wash. Blot the plate onto paper towels or other absorbent material.
 - g. Add 100 μ L of 1X Avidin-Biotin-Peroxidase Complex working solution into each well, seal the plate with a new plate seal and incubate the plate at 37°C for 30 minutes.
 - h. Wash plate five times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 - 2 minutes. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 14.6 for plate washing method).

ASSAY PROCEDURE

- i. Add 90 μL of prepared TMB color developing agent into each well, seal the plate with a new plate seal and incubate plate at 37°C in dark for 15 - 25 minutes

Note: The optimal incubation time should be determined by end user. The shades of blue should be seen in the wells with the four most concentrated Human Lipocalin-2 standard solutions; the other wells show no obvious color.

- j. Add 100 μL of prepared TMB Stop Solution into each well. The color changes into yellow immediately.
- k. Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

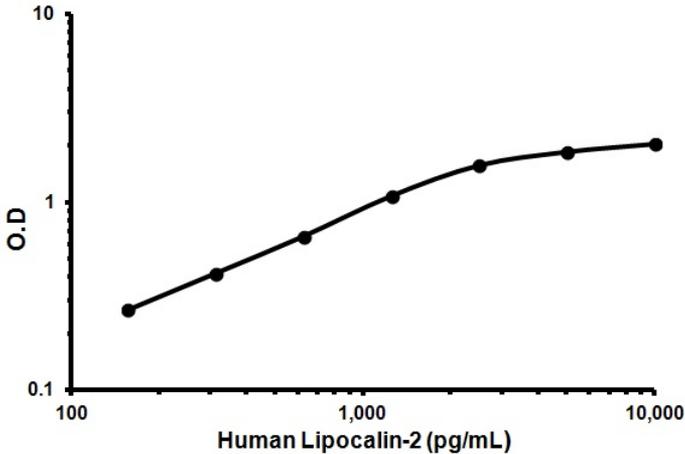
14. CALCULATIONS

For calculation, the relative O.D.450 = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human Lipocalin-2 concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, make sure to account for this in your calculations.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



| Conc. (pg/mL) | O.D. 450nm |
|---------------|------------|
| 0 | 0.075 |
| 156 | 0.267 |
| 312 | 0.418 |
| 625 | 0.657 |
| 1,250 | 1.075 |
| 2,500 | 1.563 |
| 5,000 | 1.842 |
| 10,000 | 2.028 |

16. TYPICAL SAMPLE VALUES

RANGE – 156 – 10,000 pg/mL

SENSITIVITY – < 10 pg/mL

17. ASSAY SPECIFICITY

This kit detects both endogenous and recombinant Human Lipocalin-2.

No detectable cross-reactivity with other relevant proteins.

18. TROUBLESHOOTING

| Problem | Cause | Solution |
|---|---|--|
| Poor standard curve | Inaccurate pipetting | Check pipettes |
| | Improper standards dilution | Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing |
| Low Signal | Incubation times too brief | Ensure sufficient incubation times; change to overnight standard/sample incubation |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| Samples give higher value than the highest standard | Starting sample concentration is too high. | Dilute the specimens and repeat the assay |
| Large CV | Plate is insufficiently washed | Review manual for proper wash technique. If using a plate washer, check all ports for obstructions |
| | Contaminated wash buffer | Prepare fresh wash buffer |
| Low sensitivity | Improper storage of the kit | Store the all components as directed. |

19. NOTES



For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)