# Human BD-4 ELISA Kit, pink-ONE

**1. Catalog No.** K0331272P

**2. Quantity** 96 tests

**3. Storage** 4°C

**4. Description** Human BD-4 ELISA kit contains all the necessary reagents required for performing quantitative measurement of Human BD-4 levels from samples including serum, plasma, culture medium or other biological fluids in a sandwich ELISA format.

**5. Standard range** 1000-8 pg/ml

**6. Kit Contents**

| Component | Description | Amount |
|---|---|---|
| Pre-Coated 96 well ELISA microplate | Antigen-affinity purified rabbit anti-Human BD-4 pre-coated 96well plate | 1 Plate |
| Detection Antibody (Lyophilized) | Biotinylated antigen-affinity purified rabbit anti-Human BD-4 | 2 EA |
| Standard Protein (Lyophilized) | Recombinant Human BD-4 | 2 EA |
| Color Development Enzyme | Streptavidin-HRP conjugate (600 ul) | 1 EA |
| pink-ONE Assay Diluent | 0.1% BSA in PBS (50 ml) | 1 EA |
| Prestained Color Development Reagent | pink-ONE TMB solution (10 ml) | 1 EA |
| Stop Solution | 2M $H_2SO_4$ (10 ml) | 1 EA |
| Wash Buffer (20X) | Concentrated PBST for 1L (50 ml) | 1 EA |
| Plate Sealer | | 3 EA |

**7. Reconstitution & Storage**

1. Human BD-4 Standard: 55 ng (1 vial) of recombinant Human BD-4 should be reconstituted in 55 ul sterile water for a concentration of 1 ug/ml.

2. Detection Antibody: 2.75 ug (1 vial) of biotinylated antigen-affinity purified anti-Human BD-4 should be reconstituted in 0.275 ul sterile water for a concentration of 10 ug/ml.

**Note:** Reconstituted solutions are stable at -20°C for up to 2 months. Do not repeat frozen and thawing.

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koma@komabiotech.com
Tel. 82-2-2660-5660 Fax. 82-2-2660-5669

## 8. Reagent Preparations

*** All preparations should be mixed thoroughly and warmed up at room temperature prior to use.**

1. Washing Solution (PBST): Dilute Wash Buffer at 1:20 in sterile water and mix well.

2. Pre-coated ELISA 96 well plate: Select the number of coated wells required for the assay. The remaining wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

3. Standards
   Dilute the standards and samples in pink-ONE Assay Diluent at 1:2 serial dilutions as follows:

| Step | Dilution Method | Standard conc. |
|---|---|---|
| Step A | 1 ul of Standard + 1 ml of Assay Diluent | 1000 pg/ml |
| Step B | 0.5 ml of Step A + 0.5 ml of Assay Diluent | 500 pg/ml |
| Step C | 0.5 ml of Step B + 0.5 ml of Assay Diluent | 250 pg/ml |
| Step D | 0.5 ml of Step C + 0.5 ml of Assay Diluent | 125 pg/ml |
| Step E | 0.5 ml of Step D + 0.5 ml of Assay Diluent | 62.5 pg/ml |
| Step F | 0.5 ml of Step E + 0.5 ml of Assay Diluent | 31.25 pg/ml |
| Step G | 0.5 ml of Step F + 0.5 ml of Assay Diluent | 15.62 pg/ml |
| Step H | 0.5 ml of Step G + 0.5 ml of Assay Diluent | 7.81 pg/ml |

   **Note:** Transfer 100 ul of Assay Diluent to empty well for Standard Blank.

4. Sample dilution: Dilute the samples to a proper concentration in pink-ONE Assay Diluent.

   **Note:** Dilute the samples, based on the expected concentration of the analyte, to fall within the concentration range of the standards.

5. Detection Antibody: Dilute the reconstituted detection antibody in pink-ONE Assay Diluent to a concentration of 0.5 ug/ml (1:20 dilution).

6. Color Development Enzyme: Dilute the Streptavidin-HRP conjugate 1:20 in pink-ONE Assay Diluent.





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**Tel. 82-2-2660-5660 Fax. 82-2-2660-5669**

## 9. Cautions

1. Store all solutions at 4°C and keep them from contamination.
2. All samples and kit reagents should be at room temperature (20-25°C) prior to use.
3. Vigorous washing of the plate after incubation steps is essential to obtaining low background values.
4. Dissolve antigen, standard and antibody perfectly.
5. Use clean pipet tips for each transfer to avoid cross contamination.
6. Stop solution ($H_2SO_4$) is a caustic material. Eye, hand, face, and clothing protection should be worn when handling this material.
7. Individual components of this kit contain no preservatives





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## 10. ELISA Protocol

1. Add 200 ul of Washing Solution to each well. Aspirate the wells to remove liquid and wash the plate 3 times using 300 ul of Washing Solution per well. After the last wash, invert plate to remove residual solution and blot on paper towel.

   **Note:** Do not dry the well completely and so immediately go on next step.

2. Add 100 ul of standard or sample to each well in duplicate. Cover with the Plate Sealer provided. Incubate at room temperature for at least 2 hours.

3. Aspirate the wells to remove liquid and wash the plate 4 times like as step 1.

4. Add 100 ul of the diluted detection antibody (0.5 ug/ml) per well. Cover with the Plate Sealer provided. Incubate at room temperature for 2 hours.

5. Aspirate and wash plate 4 times like as step 1.

6. Add 100 ul of the diluted Color Development Enzyme (1:20 dilute) per well. Cover with the Plate Sealer provided. Incubate 30 minutes at room temperature (or 37°C for 30 minutes).

7. Aspirate and wash plate 4 times like as step 1.

8. Add 100 ul of pink-ONE TMB Color Development Reagent to each well. Incubate at room temperature for a proper color development (1-11 minutes). pink-ONE TMB produces a deep blue color during the enzymatic degradation of $H_2O_2$ by peroxidase. To stop the color reaction, add 100 ul of the stop solution to each well.

   **Note:** Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

   **Note:** According to the reaction intensity, the color changes to violet, then deep blue during a reaction.

9. Using a microtiter plate reader, read the plate at 450 nm wavelength.





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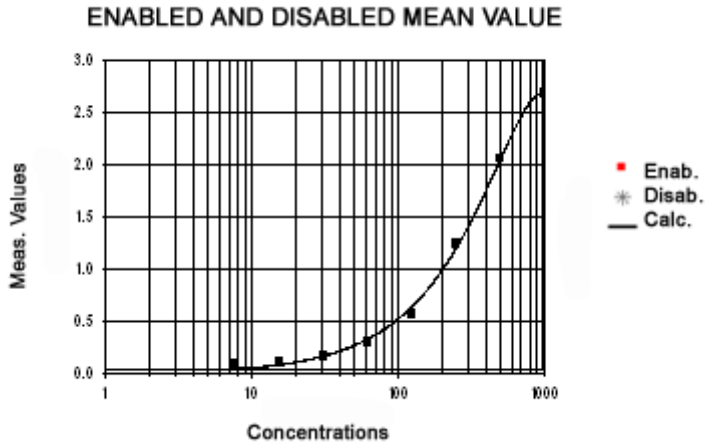
## 11. Calculation of Results

1. Average the duplicate readings from each standard, control, and samples.

2. Subtract the zero reading from each averaged value above.

3. Create a standard curve by reducing the data using ELISA reader's computer software capable of generating Standard curve-fit.
   * A standard curve should be generated for each set of samples (See example).




**Human BD-4 (pg/ml)**
**(6 minutes color development)**

## 12. Cross Reactivity

When tested at 50 ng/ml the following antigen(recombinant protein) did not exhibit significant cross reactivity:

| Host | Tested Antigen (recombinant protein) |
|---|---|
| Human | BD-1(36a.a.), BD-1(47a.a.), BD-2, BD-3 |





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## 13. Trouble shooting

| Problem | Probable Cause | Solution |
|---|---|---|
| **Low O.D.** | Reagents not fresh or contamination | Ensure reagents have been prepared correctly and are best before date. |
| | Incubation time not long enough | Ensure you are incubating the antibody for the recommended amount of time, if an incubation time is suggested. |
| | Incubation temperature too low | incubators are set in the correct temperature and working. Ensure all reagents are at room temperature before proceeding. |
| | Stop solution not added | Addition of stop solution |
| **High O.D.** | Standard reconstituted with less volume than required | Reconstitute lyophilized standard with correct volume of solution recommended in the protocol. |
| | Detection antibody, Streptavidin-HRP, Substrate solutions incubation times are too long | Decrease incubation time. |
| **Poor Duplicates** | Plate washing was not adequate or uniform | Make sure pipette tips are tightly adjusted. Confirm all reagents are removed completely in all wash steps. |
| | Not mixed well sample | Thoroughly mix samples before pipetting. |
| | Samples may have high particulate density matter | Remove the particulate matter by centrifugation. |
| | Cross-well contamination | Do not use used plate sealers<br>Do not use used pipette tips |
| **High background** | Contamination of reagents/samples | Use fresh reagents/samples and pipette carefully. |
| | Insufficient plates washing | Ensure well areas are washed adequately by filling the wells with wash buffer. |
| | Too much antibody used leading to non-specific binding | Try to use less antibody. |
| | Streptavidin-HRP too strong or left on too long exposure | Check dilution of conjugate, use it at the recommended dilution. |
| | Substrate solution or stop solution is not fresh | Use fresh substrate solution. |
| | Plate left too long before reading on the plate reader | Color will keep developing (though at a slower rate if stop solution has been added). |
| | Incubation temperature is too high | incubators are set in the correct temperature and working. |
| **Sample readings are out of range** | Samples contain no or below detectable levels of analyte or Samples contain analyte concentrations greater than the highest standard point. | If samples are below detectable levels, it may be possible to use higher sample volume. If samples are high detectable levels, it may require dilution and reanalysis. |





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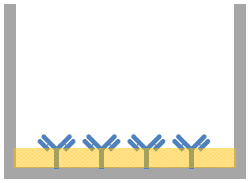
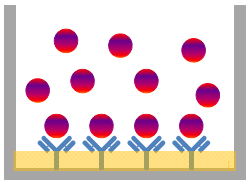
# Summary of the ELISA procedure

: Capture Antibody
: Antigen
: Detection Antibody - Biotin
: Streptavidin-HRP



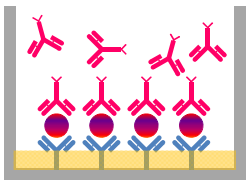
Precoated Plate

Wash the plate 4 times with PBST



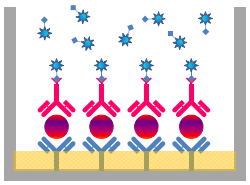
Add Standard or Samples and incubate for 2 hr at room temperature.

Wash the plate 4 times with PBST



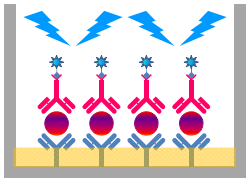
Add Detection Antibody and incubate for 2 hr at room temperature.

Wash the plate 4 times with PBST



Add Streptavidin-HRP and incubate for 30 min at room temperature.

Wash the plate 4 times with PBST



Color development with TMB substrate and read the plate at 450 nm wavelength



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## Related Products : KOMA Cytokine ELISA Kit, pink-ONE

| Cat. No. | Description |
|---|---|
| K0331251P | BD-1 Human |
| K0331208P | BD-2 Human |
| K0331254P | CNTF Human |
| K0331188P | Eotaxin-3 Human |
| K0331115P | EGF Human |
| K0331228P | EGF Mouse |
| K0331192P | bFGF Human |
| K0331226P | G-CSF Mouse |
| K0331120P | GM-CSF Human |
| K0331137P | GM-CSF Mouse |
| K0332101P | HGF Human |
| K0332112P | IGF-I Human |
| K0331225P | IGF-I Mouse |
| K0331121P | IFN-gamma Human |
| K0331138P | IFN-gamma Mouse |
| K0331209P | IFN-gamma Rat |
| K0331210P | IP-10 Human |
| K0331255P | IP-10 Mouse |
| K0331125P | IL-1 alpha Human |
| K0331141P | IL-1 alpha Mouse |
| K0331211P | IL-1 alpha Rat |
| K0331800P | IL-1 beta Human |
| K0331231P | IL-1 beta Mouse |
| K0331212P | IL-1 beta Rat |
| K0331193P | IL-2 Human |
| K0331142P | IL-2 Mouse |
| K0332100P | IL-2 Rat |
| K0331126P | IL-3 Human |
| K0331143P | IL-3 Mouse |
| K0331214P | IL-4 Human |
| K0331144P | IL-4 Mouse |
| K0331127P | IL-5 Human |
| K0331194P | IL-6 Human |
| K0331230P | IL-6 Mouse |
| K0331229P | IL-6 Rat |
| K0331215P | IL-7 Human |
| K0331216P | IL-8 Human |
| K0331232P | IL-9 Human |
| K0331123P | IL-10 Human |
| K0331213P | IL-10 Mouse |
| K0331124P | IL-12 Human |
| K0331139P | IL-12 Mouse |
| K0331235P | IL-13 Human |
| K0331201P | IL-13 Mouse |
| K0331260P | IL-15 Human |
| K0331207P | IL-17A Human |
| K0331198P | IL-17E Human |
| K0331190P | IL-20 Human |
| K0331236P | IL-21 Human |
| K0331234P | IL-22 Human |
| K0331233P | IL-31 Human |

| Cat. No. | Description |
|---|---|
| K0332133P | IL-4 Rat |
| K0331250P | Leptin Mouse |
| K0331227P | M-CSF Mouse |
| K0331195P | MIP-1 alpha Human |
| K0331202P | MIP-1 alpha Mouse |
| K0331247P | MIP-1 alpha Rat |
| K0331252P | MIP-1 beta Mouse |
| K0331217P | MIP-2 Mouse |
| K0331218P | MCP-1 Human |
| K0331219P | MCP-1 Mouse |
| K0331220P | NGF-beta Human |
| K0331191P | PDGF-BB Human |
| K0331221P | RANTES Human |
| K0331222P | RANTES Mouse |
| K0331223P | RANTES Rat |
| K0331199P | Resistin Human |
| K0331187P | sRANK Ligand Human |
| K0331203P | sRANK Ligand Mouse |
| K0331130P | SCF Human |
| K0331148P | SCF Mouse |
| K0331204P | SCF Rat |
| K0331200P | TRAIL Human |
| K0332110P | TGF-beta 1 Human |
| K0332120P | TGF-beta 2 Human |
| K0332130P | TGF-beta 3 Human |
| K0331131P | TNF-alpha Human |
| K0331186P | TNF-alpha Mouse |
| K0331196P | TNF-alpha Rat |
| K0331132P | VEGF Human |
| K0331224P | VEGF Mouse |
| K0331268P | IL-17 Mouse |
| K0332113P | Leptin Human |
| K0331263P | EGF Rat |
| K0331265P | G-CSF Human |
| K0331272P | BD-4 Human |
| K0331274P | Human BMP-2 |
| K0331281P | ICAM-1 Human |
| K0331269P | IL-21 Mouse |
| K0331261P | VEGF Rat |
| K0331267P | MCP-1 Rat |
| K0332134P | IL-10 Rat |
| K0332150P | Human sTNF RII |
| K0332132P | Rat GM-CSF |
| K0332151P | Human IL-23 |
| K0331215P | Human IL-7 |
| K0332163P | Mouse IL-9 |
| K0332142P | Mouse IL-23 |
| K0332136P | Mouse IL-5 |
| K0332144P | Mouse IL-22 |
| K0332138P | Human M-CSF |
| K0332158P | Human TGF-alpha |

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| | | | |
|---|---|---|---|
| K0331253P | IL-33 Human | K0332152P | Human BDNF |





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**koma@komabiotech.com**
**Tel. 82-2-2660-5660 Fax. 82-2-2660-5669**