PRODUCT INFORMATION & MANUAL

Human CCL20/MIP-3α ActivELISA™

NBP2-31049

For the Detection of Human CCL20/MIP-3α in the Supernatant

For research use only. Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

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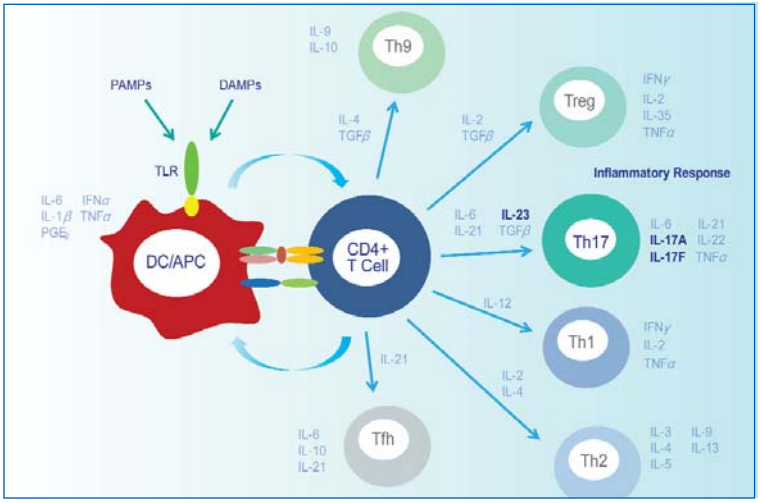
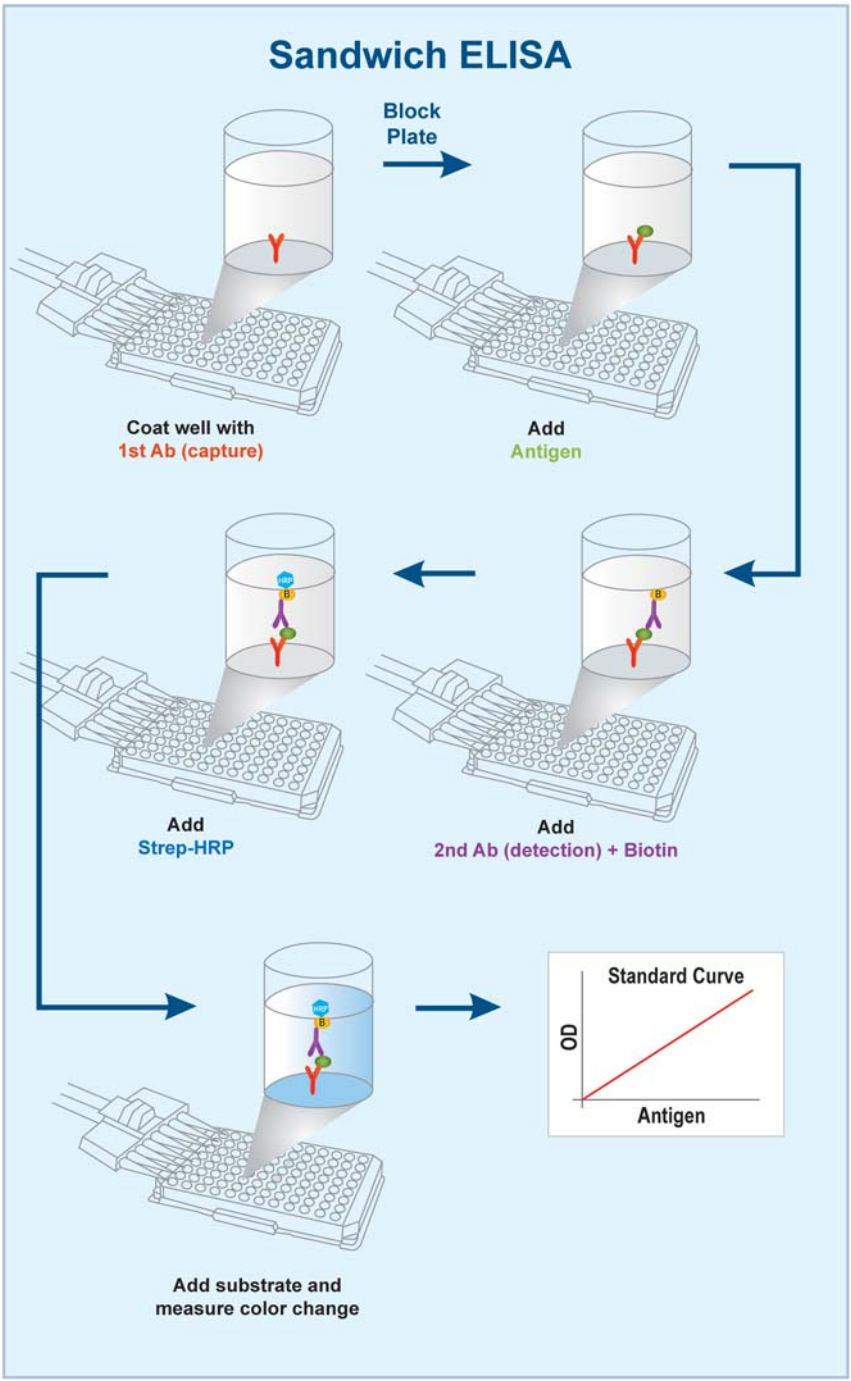
I. BACKGROUND

CCL20/MIP-3α is a small cytokine of the CC chemokine family. It acts as a chemotactic factor that attracts lymphocytes and, slightly, neutrophils, but not monocytes. It is implicated in the formation and function of mucosal lymphoid tissues via chemoattraction of lymphocytes and dendritic cells towards the epithelial cells surrounding these tissues. It is expressed constitutively by keratinocytes in epidermal layers of the skin, intestinal mucosa, epithelial crypts of tonsils, and the epithelium of Payer's patches in the intestine (Hoover DM, et al, 2002). MIP-3α triggers an adaptive immune response primarily by attracting immature dendritic cells expressing CCR6 on their surfaces to the site of inflammation, and allows the dendritic cell to take up a foreign antigen and to mature. After binding, CCR6 expression is down-regulated, causing the dendritic cell to migrate to the lymph nodes, where it binds via CCR7 and presents the antigen to CD8 T cells. Thus, it forms a bridge between innate and adaptive immune response (Serafini, et al, 2002). MIP-3α plays an immense role in various cancer entities, such as leukemia, lymphoma, melanoma, hepatocellular carcinoma, prostate cancer, colorectal adenocarcinoma and lung and oral squamous cell carcinoma (Ghadjar P, et al, 2006, 2008). MIP-3α along with its corresponding receptor CCR6 is significantly up-regulated in patients with pancreatic cancer. But inhibition of CCR6 signaling or neutralization of MIP-3α or inhibition of its production and activity may be useful in preventing further progression of the disease and may be a future basic treatment strategy in the management of pancreatic cancer (Campbell AS, et al, 2005).




Sandwich ELISA
Block Plate
Coat well with 1st Ab (capture)
Add Antigen
Add 2nd Ab (detection) + Biotin
Add Strep-HRP
Add substrate and measure color change
Standard Curve
OD
Antigen

II. OVERVIEW

The human MIP-3α ActivELISA™ Kit measures free MIP-3α in the supernatant of activated cells. Standard protocols for detecting MIP-3α activity include ELISA and Western Blot. The MIP-3α ActivELISA™ can be completed in one day using a sandwich ELISA protocol. The anti-MIP-3α antibody is coated on a plate which then captures free MIP-3α. The amount of bound MIP3-α is detected by adding a second biotinylated anti-MIP-3α antibody followed by HRP-conjugated streptavidin. ABTS substrate is then added and the concentration is determined by colorimetric detection in an ELISA plate reader.

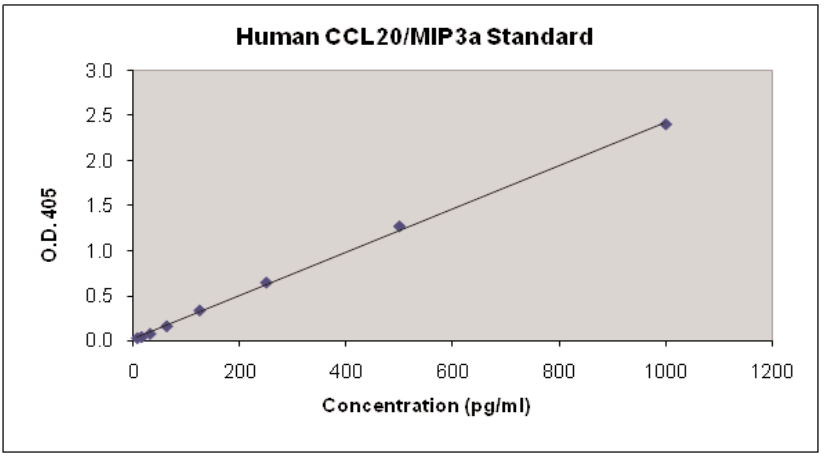
III. ADVANTAGES

- Multiple samples can be analyzed in a low-volume, high-throughput format
- Full analysis complete in just hours
- High sensitivity: Detects human MIP-3α at concentrations as low as 4 pg/ml.

IV. EXPERIMENT

EXAMPLE STANDARD

Following the provided protocol, MIP-3α standard was titrated to determine detectible levels.



V. KIT COMPONENTS AND STORAGE

Kit Components and storage for the NBP2-31049 MIP-3α ActivELISA™ Module.

REAGENTS (4°C STORAGE)		
KC-543-1	**100X Capture Antibody***	250 µl
KC-543-2	**100X Detection Antibody***	250 µl
KC-100	**BSA**	4 x 0.5 g
KC-101	**20X Wash Buffer**	50 ml
KC-141	**Coating Buffer**	25 ml
KC-142	**10X Blocking Buffer**	20 ml
KC-143	**HRP-Conjugated Streptavidin**	10 µl
KC-144	**ABTS Substrate**	2 x 12 ml
REAGENTS (-20°C, STORAGE--NON FROST-FREE FREEZER)		
KC-543-3	**Recombinant MIP-3α Standard**	1 vial, lyophilized (0.1 µg)
ADDITIONAL ITEMS INCLUDED		
ELISA Strip Well Plates		2
NBP2-31049 Manual		1

** Contains 0.02 % Sodium azide. Sodium azide is highly toxic.*

Additional items required for the ELISA (not included in the NBP2-31049 MIP-3α ActivELISA™ Module):

- 96-well ELISA plate reader

VI. PREPARATION OF REAGENTS: MIP-3α ActivELISA™

NOTE: *The included buffers and reagents are optimized for use with this kit. Substitution with other reagents is not recommended and may not give optimal results.*

1X Wash Buffer: Prepare 1X Wash Buffer by diluting 20X Wash Buffer (KC-101) in distilled water. The diluted Wash Buffer may be stored at 4°C, however we recommend preparing fresh 1X Wash Buffer for each experiment.

1X Blocking Buffer: Prepare 1X Blocking Buffer fresh prior to experiment. Dilute 10X Blocking Buffer (KC-142) with distilled water. Dissolve 0.5 g BSA (KC-100) in 50 ml of 1X Blocking Buffer in a sterile bottle.

VII. MIP-3α ActivELISA™ PROTOCOL

This kit allows for the quantitative measurement of MIP-3α in a 96-well micro titer format. All 96-wells may be used at one time or you may only use the wells as required by your experimental design. Use of duplicate wells for each time point is recommended to obtain accurate results.

APPROPRIATE CONTROLS TO INCLUDE

Following is a list of suggested controls to include with each analysis:

1. No capture antibody added to well
2. No supernatant added to well
3. No capture antibody or supernatant added to well
4. Positive control: use a sample/supernatant known to contain human MIP-3α or a recombinantly expressed human MIP-3α
5. Negative control: fresh cell culture media

ELISA PROTOCOL

Note: This protocol is designed for a full 96-well assay. You can prepare lower volumes of reagents if you are not using the entire plate for one test.

1. **Coating:** Dilute 120 µl of 100X Capture Antibody (KC-543-1) in 12 ml Coating Buffer (KC-141). Pipet 100 µl of 1X Capture Antibody into each well (A1 through H1 and A2 through H2 for the standard and any of columns 3 through 12 for your samples), seal the plate and incubate overnight (12-24 h) at room temperature. Wash the coated wells twice with 300 µl of 1X Wash Buffer.

2. **Blocking:** Add 200 µl of prepared 1X Blocking Buffer (see Preparation of Reagents, page 7) to each well to block the remaining reactive surface. Seal the plate and incubate for 30 min to 1 h at RT on a shaker set to 100 RPM.

3. **Prepare MIP-3a Standard:** Quick spin down the Recombinant MIP-3α Standard vial (KC-543-3) and add 100 µl of sterile deionized H20. Gently vortex to dissolve. Allow the vial to sit for 15 min, then vortex again. Stock standard concentration is 1 ug/ml. The stock standard may be stored in 4°C for up to 60 days after reconstitution. Suggested dilution of stock standard: add 1 ul of stock standard (1 ug/ml) in to 999 ul of 1X blocking buffer, this makes 1000 pg/ml. Set up a standard curve following the directions below:

    - Remove Blocking Buffer from wells by flicking into an appropriate waste container and gently tapping the plate face-down on paper towels. Replace with 100 µl of fresh prepared 1X Blocking Buffer in each well **B1** through **H1** and **B2** through **H2** for the standard.
    - Pipette 200 µl stock Recombinant MIP-3α Standard (1000 pg/ml) into wells **A1** and **A2**. Transfer 100 µl from wells **A1** and **A2** in to wells B1 and B2.
    - Mix wells **B1** and **B2** by pipetting.
    - Transfer 100 µl from well **B1** to **C1** and **B2** to **C2**.
    - Continue this serial dilution process to wells **G1** and **G2**. After mixing, discard 100 µl of solution from wells **G1** and **G2**.
    - Do not add standard to wells **H1** and **H2**. These will serve as blanks.

Table 1. Set up of a 96-well microtiter plate.

	Standard	Standard	Your Samples									
	1	2	3	4	5	6	7	8	9	10	11	12
A	1000 pg/ml	1000 pg/ml*	-	-	-	-	-	-	-	-	-	-
B	500 pg/ml	500 pg/ml	-	-	-	-	-	-	-	-	-	-
C	250 pg/ml	250 pg/ml	-	-	-	-	-	-	-	-	-	-
D	125 pg/ml	125 pg/ml	-	-	-	-	-	-	-	-	-	-
E	62.5 pg/ml	62.5 pg/ml	-	-	-	-	-	-	-	-	-	-
F	31.25 pg/ml	31.25 pg/ml	-	-	-	-	-	-	-	-	-	-
G	15.6 pg/ml	15.6 pg/ml	-	-	-	-	-	-	-	-	-	-
H	Blank	Blank	-	-	-	-	-	-	-	-	-	-

**Sensitivity: 1000 pg/ml is a recommended starting dilution. By reducing the starting concentration, researchers can determine the lowest detection limit of MIP-3α protein in the test samples.*

4. **Samples:** Pipet 100 µl of positive and negative controls and 100 µl test samples into the appropriate wells*. Seal the plate and incubate for 2 h at RT on a shaker. Samples may be diluted or serially diluted using 1X Blocking Buffer. **Users need to empirically determine the optimal concentrations of their test samples so that the readings fall within the curve of the protein standard.*

5. **Washing:** Remove samples and controls and wash 4X with 300 µl of 1X Wash Buffer. Tap plate several times upside down to remove residual Wash Buffer after final wash.

6. **Detecting Antibody:** Dilute 120 µl of 100X Detecting Antibody (KC-543-2) in 12 ml of 1X Blocking Buffer and add 100 µl 1X Detecting Antibody to each well. Seal the plate and incubate for 1 h at RT on a shaker.

7. **Washing:** Remove antibody solution and wash wells 4x with 300 µl of 1X Wash Buffer. Tap plate upside down to remove residual Wash Buffer after final wash.

8. **Secondary:** Dilute 2.4 µl of HRP-Conjugated Streptavidin (KC-143) in 12 ml of 1X Blocking Buffer (for one plate). Add 100 µl of diluted secondary to each well. Seal the plate and incubate for 30min at RT on a shaker.

9. Remove the secondary antibody and wash thoroughly (5X) with 300 µl of Wash Buffer letting the solution sit in wells briefly between each wash. This ensures a thorough wash and lower background. Tap plate upside down several times to remove any residual Wash Buffer.

10. **ABTS:** Add 100 µl of ABTS Substrate (KC-144) to each well. Incubate the plate at RT for 15 to 30 min. Read the color development at 405 nm.

Note: *Incubation time with ABTS Substrate may be increased or decreased depending on the concentration of samples. Most plate readers have a maximum reading of 2.0-3.0.*

VII. TROUBLESHOOTING MIP-3α ACTIVELISA™

Problem	Probable Cause	Suggestion
No signal	Failure to add all components.	Prepare a check-list and add the components in the correct order.
Low signal	Not enough supernatant per well.	Check the protein concentration. Add more Sup.
High background	Improper blocking.	Incubate with blocking buffer as recommended in the manual.
	Wells are not washed enough.	Wash plates thoroughly after each incubation.