Product Data Sheet

Biotin anti-human IL-23 (p19)

Catalog #/ Size:	511203 / 50 µg 511204 / 500 µg
Clone:	HLT2736
Isotype:	Mouse IgG1, κ
Immunogen:	Human recombinant p40 and p19 with elastin linker
Reactivity:	Human, IL-23 p19 monomer, does not cross-react with mouse IL-23
Preparation:	The antibody was purified by affinity chromatography, and conjugated with Biotin under optimal conditions. The solution is free of unconjugated Biotin.
Formulation:	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
Storage:	The antibody solution should be stored undiluted at 4° C.

Applications

Applications:	WB, IHC(Paraffin) (FPE using catalyzed signal amplification), IP, ELISA
Application Notes:	The purified HLT2736 antibody has been shown to be useful for Western blotting, immunohistochemistry using formalin-fixed, paraffin embedded tissues with catalyzed signal amplification), and immunoprecipitation.
Recommended Usage:	Each lot of this antibody is quality control tested by ELISA assay. For ELISA capture applications, a concentration range of 2-8 µg/ml is recommended. To obtain a linear standard curve, serial dilutions of IL-23 recombinant protein ranging from 4000 to 30 pg/ml are recommended for each ELISA plate. It is recommended that the reagent be titrated for optimal performance for each application.

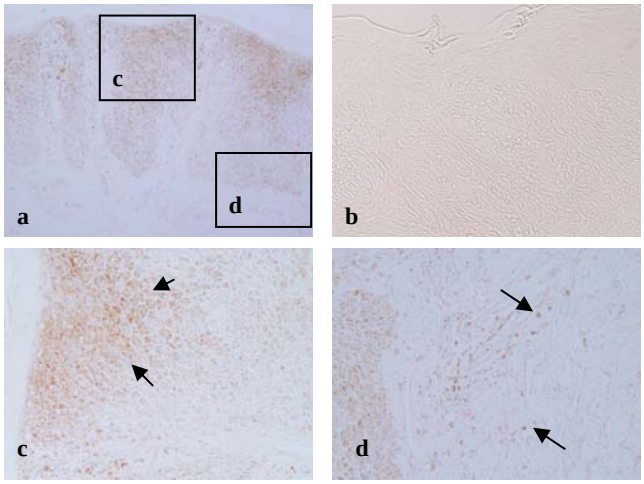

Figure 1 Immunohistochemical localization of IL-23 expression in psoriatic lesions. Paraffin embedded psoriatic lesion sections were stained with purified anti-human IL-23 (clone HTL2736, 0.5mg/ml, BioLegend, San Diego, CA) (**a, c & d)** and purified mouse IgG1,κ (clone MOPC-21, 0.5mg/ml, BioLegend, San Diego,CA) (**b)** as indicated. The brown color represents diaminobenzidene (DAB) deposition at the site of antibody binding. Low (×100, **a**) and high (×200, **b, c & d**) magnification are presented.

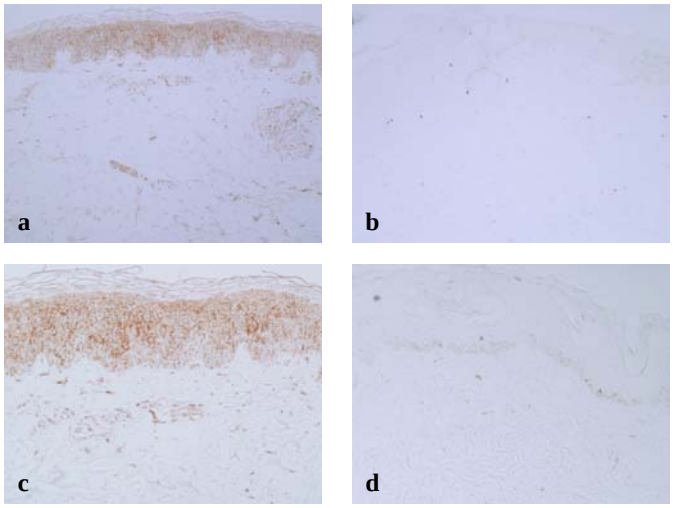

Figure 2 IL-23 highly expressed in the uninvolved skin from psoriasis patients, but not in the control normal skin. Paraffin embedded sections were stained with purified anti-human IL-23 (clone HTL2736, 0.5mg/ml, BioLegend, San Diego, CA) on the uninvolved skin from one psoriatic patient (**a & c)** and the control normal skin (**b & d**). Low (×100, a & b) and high (×200, c & d) magnification are presented.

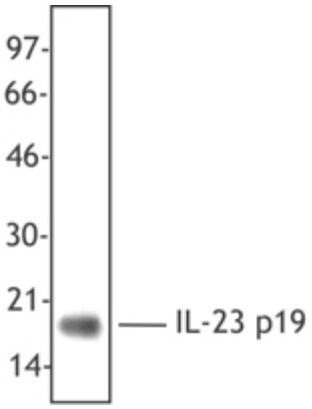

Figure 3: Linker-free, recombinant human IL-23 was resolved by electrophoresis, transferred to nitrocellulose and probed with the anti-IL23 p19 specific antibody HLT2736 antibody. Proteins were visualized using a goat anti-mouse secondary conjugated to HRP and a chemiluminescence detection system. The HLT2736 recognizes the p19-specific subunit of human IL-23. This antibody will also recognize human IL-23 (p19 +p40 subunits) in recombinant IL-23 containing a linker moiety.

Antigen Information

Other Names: p19p40, JKA3 induced upon T cell activation

Structure: Member of the IL-12 cytokine family, heterodimeric cytokine composed of p40 subunit shared with IL-12 and a specific p19 subunit

Distribution: Secreted by activated dendritic cells and macrophages

Function: Enhances IFN-γ secretion by memory (CD45RO) T cells in an IL-2 dependent manner; may induce unique Th subset (designated Th_{IL-17}) secreting IL-17, IL-6, TNF and low levels of IFN-γ in EAE model; shown to promote immunity to mycobacteria

Ligands/Receptors: IL-23 receptor (β1 subunit of IL-12 receptor and IL-23-specific subunit)

Regulation: Upregulated in viral infections such as HSV, increased in psoriatic lesions in monocytes and mature dendritic cells

Description: The HLT2736 clone recognizes human IL-23, a heterodimeric cytokine composed of the p40 subunit shared with IL-12 and a specific p19 subunit. This antibody recognizes heterodimeric IL-23, but does not react with the p40 subunit alone or recombinant IL-12 when used as a capture antibody in ELISA. IL-23 is secreted by activated dendritic cells and macrophages and has been shown to enhance IFN-γ secretion by memory (CD45RO) T cells in an IL-2 dependent manner. IL-23 may also induce unique Th subset (designated Th_{IL-17}) that secretes the cytokines IL-17, IL-6, TNF and low levels of IFN-γ. IL-23 has been shown to promote immunity to mycobacteria and has been shown to be upregulated in response to some viral infections and in psoriatic lesions. IL-23 binds to the IL-23 receptor comprised of a β1 subunit shared with the IL-12 receptor and IL-23-specific subunit. This antibody has been shown to be useful for Western blotting, immunohistochemistry using formalin-fixed, paraffin embedded tissues with catalyzed signal amplification), and ELISA as a capture antibody.

Antigen References:

1. Parham, C., *et al.,* 2002. *J. Immunol.* 168:5699.
2. Verreck, F. A., *et al.,* 2004. *Proc. Natl. Acad. Sci. USA 101:4560.*
3. Lee, E., et al., 2004. J. Exp. Med. 199:125.
4. Oppmann, B. et al., 2000. Immunity 13:715.

Protocol for IL-23 staining on paraffin embedded sections

Reagents:

1. Catalyzed Signal Amplification (CSA) System from DakoCytomation for use with mouse primary antibodies (Code K1500)

2. The CSA Ancillary System (Code K1499, DakoCytomation):
Target Retrieval Solution
Biotin Blocking Systems
TRIS-Buffered Saline with Tween 20 (TBST)(S-3306)

3. Purified anti-human IL-23 (clone HTL 2736, Cat. # 511201, 0.5mg/ml, BioLegend, San Diego, CA)

4. Purified mouse IgG1,κ (clone MOPC-21, Cat. # 400101, 0.5mg/ml, BioLegend, San Diego, CA)

Staining Procedure:

1. De-paraffinization of tissue sections: Prior to staining, deparaffinize tissue slides with xylene and gradient alcohols to remove embedding media and then re-hydrate.

2. Immerse antigen retrieval: tissue sections with Target Retrieval Solution (included in CSA Ancillary System) and microwave 1min with high power and 9 min with medium power, then cool at room temperature for 20 min.

3. Blocking of endogenous avidin-binding activity (endogenous biotin): Sequentially apply avidin and biotin to tissue sections with Biotin blocking system (included in CSA Ancillary System).

4. Staining protocol:

1) Peroxidase block: Tap off excess liquid and wipe around specimen to remove any remaining liquid and to keep reagent within the prescribed area.

Apply enough hydrogen peroxide from bottle 1 (CSA System) to cover specimen, and incubate 5 ($\pm$1) min.

Rinse gently with TBST buffer solution, and place in fresh bath for 3 min.

2) Protein block: Tap off excess liquid and wipe slide as before. Apply enough drops from bottle 2 (protein block, CSA System) to cover specimen. Incubate 5 ($\pm$1) min. Do not rinse off protein block.

3) Primary antibody or negative control reagent: Tap off excess liquid and wipe slide as before.
Apply enough IL-23 antibody [20 μg/ml (1:50)] or negative control IgG,κ to cover specimen. Incubate 15 (($\pm$1) min.
Rinse gently with TBST buffer and place in up to three fresh TBST buffer baths for 3-5 minutes each.

4) Link antibody:
Tap off excess liquid and wipe slide as before.
Apply enough drops from bottle 3 (link antibody, CSA System) to cover specimens.
Incubate 15 ($\pm$1) min.
Rinse gently with TBST buffer and place in up to three fresh TBST buffer baths for 3-5 minutes each.

5) Streptavidin-biotin complex
Tap off excess liquid and wipe slide as before.
Apply enough drops of the prepared Streptavidin-Biotin Complex (CSA System to cover specimens.
Incubate 15 ($\pm$1) min.
Rinse gently with TBST buffer and place in up to three fresh TBST buffer baths for 3-5 minutes each.

6) Amplification reagent
Tap off excess liquid and wipe slide as before.
Apply enough drops from Bottle 8 (Amplification reagent, CSA System) to cover specimens.
Incubate 15 ($\pm$1) min.
Rinse gently with TBST buffer and place in up to three fresh TBST buffer baths for 3-5 minutes each.

7) Streptavidin-peroxidase
Tap off excess liquid and wipe slide as before.
Apply enough drops from Bottle 9 (Streptavidin-Peroxidase, CSA System) to cover specimens.

Incubate 15 (±1) min.
Rinse gently with TBST buffer and place in up to three fresh TBS buffer baths for 3-5 minutes each.

8) Substrate-Chromogen solution
Tap off excess liquid and wipe slide as before.
Apply enough of the prepared substrate-Chromogen solution (CSA System) to cover specimens.
Incubate 15 (±1) min.
Rinse gently with TBST buffer and place in up to three fresh TBST buffer baths for 3-5 minutes each.

9) Mounting
Dehydrate specimens with gradient alcohol and xylene and mount with permanent mounting medium.

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