# IDO1

Concentrated and Prediluted Monoclonal Antibody
901-3210-082117

BIOCARE MEDICAL

| | | |
|---|---|---|
| **Catalog Number:** | **ACI 3210 A, B** | **API 3210 AA** |
| **Description:** | 0.1, 0.5 ml, concentrated | 6.0 ml, prediluted |
| **Dilution:** | 1:100 | Ready-to-use |
| **Diluent:** | Renoir Red | N/A |

**Intended Use:**

For In Vitro Diagnostic Use

IDO1 [UMAB126] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of IDO1 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

**Summary and Explanation:**

Indoleamine 2,3-dioxygenase 1 (IDO1) is a 403 amino acid cytoplasmic enzyme encoded by the INDO gene on human chromosome 8p22. IDO1 expression can be seen mainly in dendritic cells (DCs), such as immature and mature DCs, germinal center follicular DCs, interdigitating DCs, plasmacytoid DCs and in normal lymphoid organs, including lymph nodes, spleen, tonsils, Peyer's patches, the gut lamina propria, and the thymus (1-2). In lymph nodes, tonsils, and Peyer's patches, IDO1 staining was mainly observed in paracortical T-cell areas in cells that are larger than lymphocytes and tended to have an irregular outline, and often stellate with ramified cytoplasmic expansions. The spleen contains numerous IDO1-expressing cells in periarteriolar lymphocyte sheaths and some scattered positive cells in red pulp. The lamina propria of the duodenum, small, and large intestine contained interstitial mononuclear IDO1-positive cells, which were larger than lymphocytes. In the thymus, the expression of IDO1 was restricted to the medulla. Numerous interstitial cells, morphologically different from lymphocytes, were strongly positive, whereas a weak positivity was observed in rare epithelial cells, including Hassal's bodies (2). In the bone marrow, a weak IDO1 expression is seen in very few cells, which could correspond to some stromal or DCs (2). In non-hematolymphoid tissue, IDO1 was detected in endothelial cells of many blood vessels in the lung, the prostate and the uterus (2). Many tumor types express IDO1 protein. However, the expression is often limited to a small number of tumor cells (2). Endometrial and cervical carcinomas most frequently expressed IDO1, followed by renal cell carcinomas, nonsmall cell lung carcinomas, and colorectal carcinomas (2). IDO1 expression in human tumors is usually restricted to three different cell types: myeloid cells, endothelial cells, and tumor cells in tumor and tumor-draining lymph nodes (2-3). IDO1 expression by malignant and nonmalignant cells can inhibit T-cell immune responses, leading to immune evasion and tumor outgrowth. Based on these findings, IDO1 inhibition using epacadostat, a selective small-molecule inhibitor of IDO1, was evaluated in a mouse model of cancer and in human solid tumors (3-4). It has been found that the inhibition of IDO1 can induce T-cell dependent antitumor immunity (3-4). This activity was related to the ability of epacadostat to block regulatory T-cell activity and promote dendritic cell maturation and function. Immunohistochemical detection of IDO1 expression on tumor-infiltrating immune cells and tumor cells should be very useful in evaluating IDO1 blockade and treatment efficacy (3-4).

**Principle of Procedure:**

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

**Principle of Procedure Cont'd:**

**Source:** Mouse monoclonal
**Species Reactivity:** Human; others not tested
**Clone:** UMAB126
**Isotype:** IgG1
**Total Protein Concentration**: ~10 mg/ml. Call for lot specific Ig concentration.
**Epitope/Antigen:** Full length human recombinant protein of human IDO1
**Cellular Localization:** Membrane/cytoplasm
**Positive Tissue Control:** Tonsil
**Known Applications:**
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
**Supplied As:** Buffer with protein carrier and preservative
**Storage and Stability:**
Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

**Protocol Recommendations:**

**Peroxide Block:**
Block for 5 minutes with Biocare's Peroxidazed 1.
**Pretreatment:** Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.
**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Biocare's Background Punisher.
**Primary Antibody:** Incubate for 30 minutes at RT.
**Probe:** Incubate for 10 minutes at RT with a secondary probe.
**Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.
**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Biocare's Warp Red.
**Counterstain:**
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.
**Technical Note:**
This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.
**Performance Characteristics:**
Sensitivity and specificity on diseased tissue and tissue cross-reactivity on normal tissue is summarized in Tables 1 and 2, respectively.
**Limitations:**
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands



Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

**Limitations Cont'd:**
presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

**Quality Control:**
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

**Precautions:**
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide ($NaN_3$) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (5)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (6)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

**Troubleshooting:**
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

**References:**
1. Heitger A. Regulation of expression and function of IDO in human dendritic cells. Curr Med Chem. 2011;18:2222–33.
2. Theate I, *et al.* Extensive profiling of the expression of the indoleamine 2,3-dioxygenase 1 protein in normal and tumoral human tissues. Cancer Immunol Res. 2015; 3:161–72.
3. Beatty GL, *et al.* First-in-Human Phase I Study of the Oral Inhibitor of Indoleamine 2,3-Dioxygenase-1 Epacadostat (INCB024360) in Patients with Advanced Solid Malignancies. Clin Cancer Res. 2017; 23:3269-76.
4. Kristeleit R, *et al.* A randomised, open-label, phase 2 study of the IDO1 inhibitor epacadostat (INCB024360) versus tamoxifen as therapy for biochemically recurrent (CA-125 relapse)–only epithelial ovarian cancer, primary peritoneal carcinoma, or fallopian tube cancer. Gynecol Oncol (2017) http://dx.doi.org/10.1016/j.ygyno.2017.07.005.
5. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
6. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

**Table 1:** Sensitivity and specificity was determined by testing formalin-fixed, paraffin-embedded diseased tissues.

| Tissue | Positive Cases | Total Cases |
|---|---|---|
| Breast Cancer | 2 | 17 |
| Colon Cancer | 2 | 22 |
| Lung Squamous Cell Carcinoma | 1 | 8 |
| Lung Adenocarcinoma | 1 | 8 |
| Prostate Cancer | 1 | 14 |
| Bladder Cancer | 1 | 9 |
| Melanoma | 0 | 5 |
| GIST | 0 | 8 |

**Table 2:** Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

| Tissue | Positive Cases | Total Cases |
|---|---|---|
| Aorta | 0 | 1 |
| Bladder | 0 | 1 |
| Breast | 0 | 1 |
| Cerebellum | 1 | 1 |
| Cerebral Cortex | 0 | 1 |
| Cervix | 0 | 1 |
| Colon | 3 | 3 |
| Esophagus | 0 | 1 |
| Fallopian Tube | 1 | 1 |
| Kidney | 0 | 1 |
| Liver | 0 | 2 |
| Lung | 0 | 1 |
| Lymph Node | 1 | 1 |
| Myocardium | 0 | 1 |
| Ovary | 0 | 1 |
| Placenta | 3 | 3 |
| Prostate | 0 | 6 |
| Skeletal Muscle | 1 | 1 |
| Skin | 0 | 1 |
| Small Intestine | 2 | 2 |
| Smooth muscle | 0 | 1 |
| Spleen | 2 | 2 |
| Stomach | 0 | 1 |
| Testis | 0 | 1 |
| Thymus | 1 | 1 |
| Tonsil | 2 | 2 |
| Urethra | 0 | 1 |
| Uterus | 0 | 1 |

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE
Rev: 062117

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080