INTENDED USE AND PRESENTATION:
For in vitro diagnostic use.
AP10397, 7 ml. Prediluted antibodies in a synthetic organic linear polymer buffer solution (pH 7.4), with carrier protein and preservative for stabilisation “READY TO USE”
AP10397C, 1 ml. Concentrated antibodies with carrier protein and preservative for stabilisation.

SUMMARY, EXPLANATION AND LIMITATIONS:
Cytokeratins (CK) are intermediate filaments that constitute the cytoskeletal structure of virtually all epithelial but also of some non-epithelial cells. According to R. Moll they are divided into Type I (acidic cytokeratins, CK9 to 20) and Type II (basic cytokeratins, CK1 to 8) cytokeratins. The antibody of clone IVT-2000 (identical with CAM 5.2) was known as specific for CK8 and CK18 so far. Recent investigations pointed out that the antibody (like CAM 5.2) reacts with CK7 (48 kDa) and CK8 (52 kDa). The antibody detects most epithelial tissues and developed tumours. However, stratified epithelia and some non-stratified squamous cell carcinomas are negative for anti-Cytokeratin 8/18. Immunohistochemistry (IHC) is a complex technique in which immunological and histological detection methods are combined. In general, the manipulation and processing of tissues before immunostaining, especially different types of tissue fixation and embedding, as well as the nature of the tissues themselves may cause inconsistent results (Nadj and Morales, 1983). Endogenous pseudoperoxidase and peroxidase activity or endogenous biotin and alkaline phosphatase activity can cause non-specific staining results depending on the detection system used. Tissues that contain Hepatitis B surface antigen (HBsAg) can produce false positives when using HRP detection systems (Omata et al., 1980). Insufficient contrast staining and/or improper mounting of the sample may influence the interpretation of results.

Isotype: IgG2a/kappa
Immunogen: Human carcinoma cell line HT29.
Staining pattern: Cytoplasmic.
The interpretation of the stain results is the full responsibility of the user. Any experimental result must be confirmed by a medically established diagnostic product or procedure.
Positive control: Tissue sample from skin or colon adenocarcinoma.
External negative control: Tissue sample homologous to the test sample incubated with an antibody isotype not specific for cytokeratin 8/18.

APPLICATIONS:
This antibody is designed for the specific localization of human cytokeratins 8 and 18 using IHC techniques in formalin-fixed, paraffin-embedded tissue sections. This antibody is useful for identifying normal and neoplastic cells of epithelial origin in histological sections. Stain normal epithelium of the ducts and are positive in adenocarcinomas of the breast, intestinal, hepatic, renal, neuroendocrine carcinomas (eg Merkel cell tumor), mesotheliomas, germ cell tumors (except seminoma), synovial sarcoma and epithelioid . Some tissues of epithelial origin can be immunostained as smooth muscle, some breast sarcomas, melanomas (in cryostat sections), malignant meningiomas and neuroblastomas.

PRODUCT COMPOSITION:
Mouse immunoglobulin IgG2a/kappa, clone IVT-2000, obtained from culture supernatant. The preparation contains saline buffer, stabilising and carriers proteins, and sodium azide as a preservative.

METHODS AND PROCEDURE:
Principles of the procedure: The demonstrations of antigens by IHC is a sequential procedure with several steps involving first the application of a specific antibody for the antigen of interest (primary antibody), then a secondary antibody which joins to the first, an enzyme complex, and the addition of a chromogenic substrate. The sample is washed between each step. Enzymatic activation of the chromogenic substrate creates a visible product where the antigen is located. The results are interpreted using a light microscope. The primary antibody can be used both in manual IHC and with automated immunostainers.

Specimen: Paraffin-embedded tissue samples should be used. Western blot techniques are not recommended.

Staining procedure:
Antigen retrieval HIER Citrate Buffer pH 6.5
Working dilution (only for concentrates) 1:25 – 1:100
Incubation 30 min; RT
Control Tissue Skin, colon adenocarcinoma

Amplification and development of the immunostaining: Follow standard procedure and the recommendations given by the manufacturer for the materials used. In the case of using automated immunostainers, use the specified buffers and materials for each instrument.

See our web site at www.gennova-europe.com for detailed protocols ancillary reagents and support products.

REQUIRED MATERIALS BUT NOT SUPPLIED:
All reagents, materials, and laboratory equipment for IHC procedures are not provided with this antibody. This includes adhesive slides and cover slips, positive and negative control tissues, Xylene or adequate substitute, ethanol, distilled H2O, heat pretreatment equipment (pressure cooker, steamer, microwave), pipettes, Coplin jars, glass jars, moist chamber, histological baths, negative control reagents, counter-staining solution, mounting materials, and microscope. Buffered solutions for antigen retrieval, enzyme treatments, highly sensitive detection systems, and other auxiliary reagents are available from Gennova Scientific.
Anti-Cytokeratin 8/18 (Clone: IVT-2000; like CAM5.2)
Mouse monoclonal antibody
Reference: AP10397; AP10397C

STORAGE AND STABILITY:
Store at 2-8 °C until the expiration date printed on product label. Do not use after the expiration date. If fresh solutions are required, these must be prepared immediately prior to use, and will be stable for at least one day at room temperature (20-25°C). Unused portion of antibody preparation should be discarded after one day. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user. The validity period of the ready to use products when opened, is the same as the expiration date indicated on the label of intact product.
Gennova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and used as recommended. No other guarantees are provided. Under no circumstances is Gennova Scientific obliged to cover damages caused by use of this reagent.

TROUBLESHOOTING:
If unusual staining is observed or any other deviations from the expected results, please read these instructions carefully, along with the instructions from the detection system. If this does not solve the problem, please contact Gennova Scientific’s technical support department or your local distributor.

PRECAUTIONS:
Use only by qualified personnel.
Use proper protective equipment in order to avoid contact with reagents and samples in the eyes, skin, and mucosal tissues. In case of contact with sensitive areas, immediately flush the affected area with water. Avoid microbial contamination of the reagent, as this may produce nonspecific staining results. This antibody contains sodium azide (NaCN), used as a stabilising agent, which is not considered to be a hazardous material in the concentration used. Concentration of sodium azide in drainage pipes made of lead or copper can cause the formation of highly explosive metallic azides. In order to avoid this, sodium azide must be disposed of along with a large volume of running water. Material safety data sheet (MSDS) for pure sodium azide is available upon request.

PERFORMANCE CHARACTERISTICS:
Gennova Scientific has performed studies to evaluate the functioning of these antibodies for use with standard detection systems, concluding that the product is both specific and sensitive for the antigen of interest.

BIBLIOGRAPHY:
Bishop P. www.e-immunohistochemistry.com

10 × 20 ×
IHC of Cytokeratin 8/18 (IVT-2000) on FFPE colon carcinoma tissue.
Citrate pH 6.5; DAB; Hematoxylin