TTF-1 + CK5/6 Cocktail
Mouse anti-TTF-1 and Mouse anti-Cytokeratin 5/6
Cat. No.: CO004K (1 ml Concentrate); CO004K-05 (0.5 ml Concentrate); COG004 (6 ml Ready-to-use)

Instructions for use

Intended use
This antibody cocktail is designed for the specific localisation of TTF-1 as well as Cytokeratins 5 and 6 in formalin-fixed, paraffin-embedded tissue sections. TTF-1 + CK5/6 Cocktail is intended for research use only.

Specifications

Specificity:	TTF-1, Cytokeratin 5 and Cytokeratin 6
Immunogens:	TTF-1: recombinant protein according to rat TTF-1 Cytokeratin 5/6: purified Cytokeratin 5
Clones:	8G7G3/1 (TTF-1) and D5/16B4 (Cytokeratin 5/6)
Isotypes:	Mouse IgG1κ (TTF-1) and mouse IgG1κ (Cytokeratin 5/6)
Species reactivity:	human+, others not tested

Summary and description

Thyroid transcription factor-1 (TTF-1), also known as *thyroid-specific enhancer-binding protein* (T/EBP), is a 40 kDa protein which regulates genes specifically expressed in thyroid glands and lungs. TTF-1 is a selective marker for adenocarcinomas of these tissues where it is located in the nuclei of epithelial cells. Other organs are negative for TTF-1. Detection of TTF-1 is useful to discriminate adenocarcinomas of the lung from metastatic breast carcinomas and mesotheliomas.
The antibody of clone D5/16B4 detects basic high-molecular CK5 (58 kDa) and CK6 (56 kDa). In Western Blot it also reacts weakly with CK4. CK5 is expressed in basal, intermediate and superficial layers of stratified epithelia, transitional and complex epithelia and mesotheliomas. In complex epithelia CK5 is detectable in basal cells. Only a few simple epithelia and non-epithelial cells express CK5. CK6 is expressed by proliferating squamous epithelia and is often paired with CK16 (48 kDa). Important applications of CK5/6-antibodies are the discrimination of poorly differentiated squamous cell carcinomas (mostly positive) from adenocarcinomas (mostly negative), of mesotheliomas (positive) from lung carcinomas (negative) as well as the differential diagnosis of atypical proliferations of the breast.
The combination of antibodies against TTF-1 and cytokeratin 5/6 can be useful for the discrimination of mesotheliomas from adenocarcinomas of the lung in only one tissue section.

Reagent provided
Mixture of mouse monoclonal antibodies in buffer with carrier protein and preservative for stabilisation in the formats:

Concentrate:	1 ml	(Cat. No. CO004K)
Concentrate:	0.5 ml	(Cat. No. CO004K-05)
Ready-to-use:	6 ml	(Cat. No. COG004)

Dilution of primary antibody
Dilution of Zytomed Systems' concentrated antibody depends on the detection system used. The final working dilution must always be determined by the user. The elaboration of staining protocol should be done by an experienced specialist. For Zytomed Systems' recommendations see chapter 'Staining procedure'.

Storage and handling
The antibody cocktail should be stored at 2-8°C without further dilution.
If necessary, dilutions of the antibody should be done in a suitable antibody dilution buffer (e.g. ZUC025 from Zytomed Systems). The diluted antibody should be stored at 2-8°C after use. Stability of this working solution depends on various parameters and has to be confirmed by appropriate controls.
The antibody cocktail provided is suitable for use until the expiry date indicated on the label, if stored at 2-8°C. Do not use product after the expiry date. Positive and negative controls should be run simultaneously with all specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Zytomed Systems' technical support or your local distributor.

Precautions
Use through qualified personnel only.
Wear protective clothing to avoid contact of reagents and specimens with eye, skin and mucous membranes. If reagents or specimens come in contact with sensitive area, wash with large amounts of water.
Microbial contamination of the reagent must be avoided, since otherwise non-specific staining may occur.
Sodium azide (NaN_3), used for stabilisation, is not considered hazardous material in the concentration used.
Reaction of sodium azide with lead or copper in drainage pipes can result in the formation of highly explosive

metallic azides. Sodium azide should be discarded in a large volume of running water to avoid formation of deposits. Material safety data sheets (MSDS) are available upon request.

Staining procedure

Refer to the following table for conditions specifically recommended for this antibody. Also refer to detection system data sheets for guidance on specific staining protocols or other requirements.

Parameters	Zytomed Systems recommendations
*Pre-treatment:	Heat Induced Epitope Retrieval in Citrate Buffer pH 6.0 (ZUC028)
*Control tissue:	for TTF-1: thyroid gland, lung, adenocarcinoma of lung for Cytokeratin 5/6: mesothelioma, prostate
*Working dilution:	1:50 (for concentrated antibodies only)
*Incubation time:	60 minutes

Quality control

The recommended positive control tissues for this antibody cocktail are thyroid gland, lung tissue, or adenocarcinoma of lung for TTF-1 as well as mesothelioma and prostate for CK5/6 respectively. We recommend carrying out a positive and a negative control with every staining run. Please refer to the instructions of the detection system for guidance on general quality control procedures.

Troubleshooting

If you observe unusual staining or other deviations from the expected results please read these instructions carefully, refer to the instructions of the detection system for relevant information or contact your local distributor.

Expected results

The antibody against TTF-1 stains positive in nuclei of normal and pathologic tissue of thyroid glands and lung. The antibody against cytokeratins 5 and 6 stains positive in the cytoplasm of cytokeratin 5 and cytokeratin 6 positive cells. Interpretation of the staining results is solely the responsibility of the user. Any experimental result should be confirmed by a medically established diagnostic procedure.

Limitations of the Procedure

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining, for example variations in fixation and embedding or the inherent nature of the tissue can cause inconsistent results (Nadji and Morales, 1983). Endogenous peroxidase, alkaline phosphatase or biotin may cause non-specific staining depending on the detection system used. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive results with HRP (horse radish peroxidase) detection systems (Omata *et al*, 1980). Inadequate counterstaining and mounting can influence the interpretation of the results.

Zytomed Systems warrants that the product will meet all requirements described from its shipping date until the expiry date is reached, if the product is stored and utilised as recommended. No additional guarantees can be given. Under no circumstances shall Zytomed System be liable for any damages arising out of the use of the reagent provided.

Performance characteristics

Zytomed Systems has conducted studies to evaluate the performance of the antibody for use with a standard detection system. The product has been found to be sensitive and specific to the antigen of interest with minimal or no cross-reactivity.

Bibliography:

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Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-9, 1983
Omata M et al. Am J Clin Pathol 73(5): 626-32, 1980

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Explanations of the symbols on the product label:

REF	Bestellnummer Catalog Number Reference du catalogue		Verwendbar bis Use By Utiliser jusque		Gebrauchsanweisung beachten Consult Instructions for use Consulter les instructions d'utilisation
LOT	Chargenbezeichnung Batch Code Code du lot		Lagerungstemperatur Temperature Limitation Limites de température	RUO	Nur für Forschungszwecke For Research Use Only Pour la recherche uniquement
IVD	In vitro Diagnostikum In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro		Achtung Warning Attention		Hersteller / Manufacturer / Fabricant Zytomed Systems GmbH • Anhaltinerstraße 16 14163 Berlin, Germany • Tel: (+49) 30-804 984 990 www.zytomed-systems.com