

PIN-Cocktail

Rabbit anti-P504S (AMACR) and Mouse anti-p63

Cat. No.: CO001K (1 ml Concentrate); CO001K-05 (0.5 ml Concentrate);
COG001 (6 ml Ready-to-use)

Instructions for use

Intended use

This antibody cocktail is designed for the specific localisation of human P504S (also known as AMACR or α -Methylacyl-CoA-Racemase) and of human p63 in formalin-fixed, paraffin-embedded tissue sections. This PIN-Cocktail is intended for research use only.

Specifications

Specificity:	P504S (AMACR) and p63
Clone:	Polyclonal (P504S) and Y4A3 (p63)
Isotype:	Rabbit IgG (P504S) and mouse IgG2a (p63)
Species reactivity:	Human +, others not tested

Summary and Description

P504S or AMACR (α -Methylacyl-CoA-Racemase) is an enzyme, which is involved in β -oxidation of branched-chain fatty acids. It is expressed in prostatic adenocarcinomas but not in benign glands. Therefore, detection of P504S is helpful in distinguishing malignant from benign prostate glands. P504S is also detectable in most cases of HGPIN (high-grade prostatic intraepithelial neoplasia). Additionally, approximately 15-20% of AAH (atypical adenomatous hyperplasia) are expressing the enzyme. Recent investigations (A. Nassar et al. 2005) have shown that P504S is detectable in different levels in certain other tumours like colon, lung, endometrial and breast carcinomas as well as melanomas. Urothelial, kidney and liver cell carcinomas are also sometimes positive.

p63 is detectable in benign basal cells of the prostate and in other epithelial tissues. Malignant tumours of prostate are negative for p63.

Combined detection of P504S and p63 is very useful for the diagnosis of small prostate cancers in needle biopsies or HGPIN. Anti-P504S antibody stains positive and granular in cytoplasm of malignant and pre-malignant prostate glands whereas anti-p63 antibody stains positive in nuclei of benign glands.

Reagent provided

Mixture of rabbit polyclonal antibody and mouse monoclonal antibody in buffer with carrier protein and preservative for stabilisation in the format:

Concentrate:	1 ml (Cat. No. CO001K)
Concentrate:	0.5 ml (Cat. No. CO001K-05)
Ready-to-use:	6 ml (Cat. No. COG001)

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.

Dilutions of the concentrated antibody should be done with 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) (Option: <i>HIER with EDTA pH 9.0, which increases signal intensity but often leads to higher background</i>)
*Control tissue	Prostate carcinoma
*Working dilution	1:50 (for concentrated antibodies only)
*Incubation time	30 minutes

For visualisation of both antibodies in the cocktail the detection system must be compatible with primary antibodies from rabbit and mouse. A two-colour staining is also possible when using suitable double staining detection reagents (e.g. POLDS-006).

Quality control

The recommended positive control tissue for this antibody cocktail is prostate carcinoma. 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 P504S stains positive in the cytoplasm of epithelial cells. Staining is granular. The antibody against p63 stains positive in nuclei of epithelial 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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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		Hersteller / Manufacturer / Fabricant Zytomed Systems GmbH Anhaltinerstraße 16 14163 Berlin, Germany Tel: (+49) 30-804 984 990 www.zytomed-systems.de
IVD	In vitro Diagnostikum In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro	RUO	Nur für Forschungszwecke For Research Use Only Pour la recherche uniquement		