

# Pan-B-Cocktail Mouse anti-CD20 and Rabbit anti-Pax-5 Cat. No.: CO002K (1 ml Concentrate); CO002K-05 (0.5 ml Concentrate); COG002 (6 ml Ready-to-use)

## Instructions for use

## Intended use

This antibody cocktail is designed for the specific localisation of human B-cells in formalin-fixed, paraffin-embedded tissue sections. Pan-B-Cocktail is intended for research use only.

Specifications	
Specificity:	CD20 and Pax-5
Immunogens:	Pax-5: synthetic peptide according to the C-terminus of human Pax-5 protein
-	CD20: B-cells derived from human tonsil
Clone:	L26 (CD20) and polyclonal (Pax-5)
Isotypes:	Mouse IgG2a (CD20) and rabbit Ig (Pax-5)
Species reactivity:	human+, others not tested

## Summary and description

Anti-CD20 antibody reacts with a formalin-resistant epitope of the CD20-antigen which is found on most B-cells. CD20 is therefore called pan-B-cell marker. The antibody of clone L26 is believed to be the best and most specific pan-B-cell antibody. L26 shows no cross-reactivity with non-lymphatic tissue.

Pax-5 (Paired Box Protein 5), also known as BSAP (B-Cell Specific Activator Protein), is a nuclear marker for Blymphocytes. The protein is detectable already in pro- and pre-B-cells as well as in mature B-cells. Its expression is down-regulated in plasma cells. Pax-5 affects the expression of certain B-cell specific markers, such as CD19 and CD20.

Recent investigations have shown that detection of Pax-5 has in some cases a higher specificity and sensitivity for B-cells than detection of CD20 or CD79a. This holds true e.g. for DLBCL (diffuse B-cell large cell lymphomas). In addition new therapies targeting CD20 are expected to lead to increased numbers of CD20 negative B cell lymphoma and leukaemia after therapy (Clarke LE et al. J Cutan Pathol 30:459-462, 2003). In these cases alternative markers like Pax-5 can be helpful.

The combined staining of CD20 and Pax-5 leads to a reliable detection of B-cells particularly in this context. Anti-CD20 antibody stains positive in the membrane and sometimes in the cytoplasm of B-lymphocytes. Anti-Pax-5 stains positive in the nuclei.

## **Reagent provided**

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

Concentrate:	1 ml	(Cat. No. CO002K)
Concentrate:	0.5 ml	(Cat. No. CO002K-05)
Ready-to-use:	6 ml	(Cat. No. COG002)

## **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 wit hout further dilution.

If necessary, dilutions of the antibody cocktail 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 wor king 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. ProClin300 is used for stabilisation. 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 (for example in Citrate Buffer pH 6.0 ZUC028)
	(Alternative: EDTA pH 9.0, which leads to stronger signals but sometimes background staining)
*Control tissue:	Tonsil
*Working dilution:	1:50 (for concentrated antibodies only)
*Incubation time:	30 - 60 minutes

For visualisation of both antibodies in the cocktail the detection system must be compatible with primary antibodies from rabbit <u>and</u> 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 is tonsil. 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 CD20 stains positive in the cell membrane, sometimes also in the cytoplasm of B-cells. The antibody against Pax-5 stains positive in nuclei of B-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:

Torlakovic E et al. Am J Surg Pathol 26:1343-1350, 2002 Tiacci E et al. Cancer Res 64:7399-7404, 2004 Clarke LE et al. J Cutan Pathol 30:459-462, 2003 Stein H. Immunität und Infektion, 4:52-69, 95-109, 1976. 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

October 15, 2013

Rev: A1013

Doc: DBE\_CO002K\_CO002K-05\_COG002

Explanations of the symbols on the product label:

REF	Bestellnummer Catalog Number Reference du catalogue	Verwendbar bis Use By Utiliser jusque	<u>[]i</u>	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	