

# OX-40/CD134 Control Slides



## Intended Use

For In Vitro Diagnostic Use.

## Summary and Explanation

OX-40 also known as CD134 and Tumor Necrosis Factor Receptor Superfamily Member 4 (TNFRSF4), is a member of the TNFR-superfamily of receptors which is not constitutively expressed on activated CD4 and CD8 T cells as well as a number of other lymphoid and non-lymphoid cells, unlike CD28. OX40 and its binding partner, OX40L (CD252), are costimulatory signals from OX40 to a conventional T cell to promote division and survival, augmenting the clonal expansion of effector and memory populations as they are being generated to antigen. OX40 and OX40L also regulate cytokine production from T cells, antigen-presenting cells, NK cells, and NKT cells, and modulate cytokine receptor signaling. In line with these important modulatory functions, OX40/OX40L interactions have been found to play a central role in the development of multiple inflammatory and autoimmune diseases. Besides the essential role played by OX40 signaling in generating memory CD4 T cells, recent reports show that it also has a unique role in generating memory CD8 T cells. In addition, recent genome-wide association studies have identified single-nucleotide polymorphisms of the OX40L and OX40 genes that are related to cardiovascular diseases and SLE, providing direct evidence for the involvement of the OX40-OX40L interaction in human diseases.

OX40 is a potent costimulatory receptor that can potentiate T-cell receptor signaling on the surface of T lymphocytes, leading to their activation by a specifically recognized antigen. In particular, OX40 engagement by ligands present on dendritic cells dramatically increases the proliferation, effector function, and survival of T cells. Preclinical studies have shown that OX40 agonists increase antitumor immunity and improve tumor-free survival by increasing T and B cell responses to reporter antigen immunizations, led to preferential upregulation of OX40 on CD4(+) FoxP3(+) regulatory T cells in tumor infiltrating lymphocytes, and increased the antitumor reactivity of T and B cells in patients with melanoma.

## Presentation

Five slides of OX-40/CD134 positive tissues, each mounted on a Hydrophilic Plus Slides, provided in a plastic mailer.

Catalog No.	Quantity
BSB-9318-CS	5 slides
BSB 3126	5 slides

**Storage** Store at 20-25°C

## Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
4. Dispose of unused solution with copious amounts of water.
5. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information, refer to Safety Data Sheet for this product.
9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

## Stability

**This product is stable up to the expiration date on the product label.**

Do not use after expiration date listed on package label.

## IHC Protocol

1. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
2. Any of three heating methods may be used:

### a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.

### b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.

### c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 30-60 minutes.

3. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
4. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
5. Wash slides with ImmunoDNA washer or DI water.
6. Continue IHC staining protocol. Wash slides between each step with ImmunoDNA washer solution.

### Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP
Peroxidase/AP Blocker	5 min.	5 min.	5 min
Primary Antibody	30-60 min.	30-60 min.	30-60 min.
1st Step Detection	10 min.	30-45 min.	15 min.
2nd Step Detection	10 min.	Not Applicable	15 min.
Substrate- Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain / Coverslip	Varies	Varies	Varies

### Abbreviated IF Protocol

Step	Incubation Time
Rinse slides in IF wash buffer	5 minutes
Drain and wipe excess IF wash buffer off slide	
Conduct remaining steps in the dark	
Apply Antibody	30-60 minutes
Rinse with 3 changes of IF wash buffer	3x15 minutes each
Coverslip with IF mounting medium	

### Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMunter (BSB 0169-0174) or organic solvent based resin such as PermaMunter (BSB 0094-0097), refer to PI0174 or PI0097.

### Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized, and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

### References

1. Kawamata S, Hori T, Imura A, Takaori-Kondo A, Uchiyama T. Activation of OX40 signal transduction pathways leads to tumor necrosis factor receptor-associated factor (TRAF) 2- and TRAF5-mediated NF-kappaB activation. J. Biol. Chem.1998; 273 (10): 5808-14.
2. Michael Croft, et al. The Significance of OX40 and OX40L to T cell Biology and Immune Disease. Immunol Rev. 2009; May; 229(1): 173-191.
3. Ishii N, et al. OX40-OX40 ligand interaction in T-cell-mediated immunity and immunopathology. Adv Immunol. 2010; 105:63-98.
4. Curti BD, et al. OX40 is a potent immune-stimulating target in late-stage cancer patients. Cancer Res. 2013 Dec 15;73(24):7189-98.
5. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012.  
<https://www.cdc.gov/mmwr/pdf/other/su6101.pdf>

### Symbol Key / Légende des symboles/Erläuterung der Symbole

<b>EC</b> <b>REP</b>	QAdvis EAR AB Ideon Science Park Scheelevägen 17 SE-223 70 Lund, Sweden	 Storage Temperature Limites de température Zulässiger Temperaturbereich	 Manufacturer Fabricant Hersteller	<b>REF</b>	Catalog Number Référence du catalogue Bestellnummer
<b>IVD</b>	In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	 Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	 Expiration Date Utiliser jusqu'à Verwendbar bis	<b>LOT</b>	Lot Number Code du lot Chargenbezeichnung