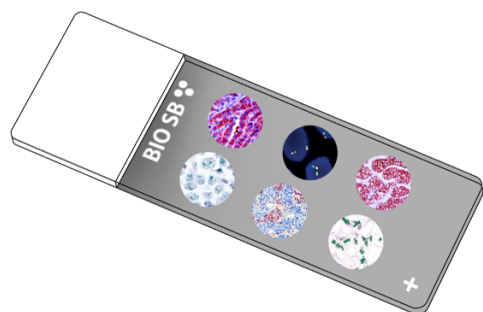


# TORC3/CRTC3 Control Slides



## Intended Use

For In Vitro Diagnostic Use.

## Summary and Explanation

CREB-Regulated Transcription Coactivator 3 (CRTC3) or TORC3, is a transcriptional coactivator for cAMP-responsive element-binding protein 1 (CREB1), which activates transcription through both consensus and variant CRE sites. It also regulates the expression of specific CREB-activated genes such as the steroidogenic gene, StAR. TORC3 works as a potent coactivator of PPARGC1A and inducer of mitochondrial biogenesis in muscle cells. It is a coactivator for TAX activation of the human T-cell leukemia virus type 1 (HTLV-1) long terminal repeats (LTR). TORC/CRTC proteins help regulate the cell's response to c-AMP signaling, and are thus important activators in metabolic networks. Gain-of-function mutations can contribute to cancer cell survival and proliferation, such as in Melanoma, where TORC3/CRTC3 overexpression contributes to invasiveness and poorer prognosis. TORC3/CRTC3 is also involved in melanogenesis and the homeostasis of protecting against UV damage while allowing production of Vitamin D. Metabolic pathway regulation also contributes to the management of conditions such as Diabetes and cancer cachexia. CRTC-MAML2 gene fusions have been identified in 30-60% of Mucoepidermoid Carcinomas in the salivary glands, though no prognosis was found from fusion status.

## Presentation

Five slides of TORC3/CRTC3 positive tissues, each mounted on Hydrophilic Plus Slides, provided in a plastic mailer.

Catalog No.	Quantity
BSB-9443-CS	5 slides

**Storage** Store at 20-25°C

## Precautions

- For professional users only. Results should be interpreted by a qualified medical professional.
- Ensure proper handling procedures are used with this reagent.
- Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
- Dispose of unused solution with copious amounts of water.
- Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).

8. For additional safety information, refer to the 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 PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (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. Manna PR, et al. Transcriptional regulation of the mouse steroidogenic acute regulatory protein gene by the cAMP response-element binding protein and steroidogenic factor 1. *J Mol Endocrinol.* 2003;30.3: 381-397.
2. Altarejos J, et al. CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. *Nat Rev Mol Cell Biol.* 2011;12.3: 141-151.
3. Than TA, et al. Role of cAMP-responsive element-binding protein (CREB)-regulated transcription coactivator 3 (CRTC3) in the initiation of mitochondrial biogenesis and stress response in liver cells. *J Biol Chem.* 2011;286.25: 22047-22054.
4. Wu Z, et al. Transducer of regulated CREB-binding proteins (TORCs) induce PGC-1 $\alpha$  transcription and mitochondrial biogenesis in muscle cells. *PNAS.* 2006; 103.39: 14379-14384.
5. Berdeaux R, et al. Anabolic and Pro-metabolic Functions of CREB-CRTC in Skeletal Muscle: Advantages and Obstacles for Type 2 Diabetes and Cancer Cachexia. *Front Endocrinol (Lausanne).* 2019;10:535.
6. Siu Y, et al. TORC1 and TORC2 coactivators are required for tax activation of the human T-cell leukemia virus type 1 long terminal repeats. *J Virol.* 2006;80(14):7052-7059.
7. Yoo H, et al. CRTC3, a sensor and key regulator for melanogenesis, as a tunable therapeutic target for pigmentary disorders. *Theranostics.* 2021;11(20):9918-9936.
8. Ostojic J. et al. Transcriptional co-activator regulates melanocyte differentiation and oncogenesis by integrating cAMP and MAPK/ERK pathways. *bioRxiv.* 2020.
9. Birkeland A, et al. Correlation of CRTC1/3-MAML2 fusion status, grade, and survival in Mucoepidermoid Carcinoma. *Oral Oncol.* 2018;68:5-8.

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

 QAdvis EAR AB Ideon Science Park Scheelevägen 17 SE-223 70 Lund, Sweden	 Storage Temperature Limites de température Zulässiger Temperaturbereich	 Manufacturer Fabricant Hersteller	 Catalog Number Référence du catalogue Bestellnummer
 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	 Lot Number Code du lot Chargenbezeichnung