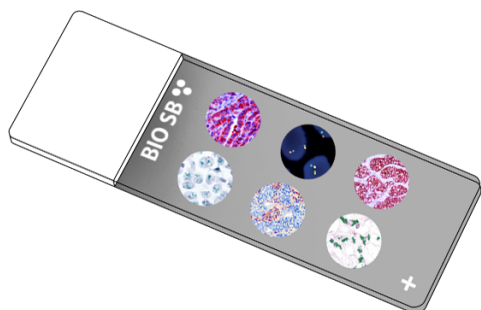


CITED1 Control Slides



Intended Use

For In Vitro Diagnostic Use.

Summary and Explanation

CITED1 is a transcriptional coactivator of the p300/CBP-mediated transcription complex. It enhances SMAD-mediated transcription by strengthening the functional link between the DNA-binding SMAD transcription factors and the p300/CBP transcription coactivator complex. It also stimulates estrogen-dependent transactivation activity mediated by estrogen receptor signaling by stabilizing the interaction of estrogen receptor ESR1 and histone acetyltransferase EP300. CITED1 also positively regulates TGF-beta signaling through its association with the SMAD/p300/CBP-mediated transcriptional coactivator complex, and associates with chromatin to the estrogen-responsive TGF-alpha promoter region in an estrogen-dependent manner. Additionally, CITED1 induces transcription from estrogen-responsive promoters and protection against cell death in Breast Cancer, potentiates EGR2-mediated transcriptional activation activity from the ERBB2 promoter, and acts as an inhibitor of osteoblastic mineralization through a cAMP-dependent parathyroid hormone receptor signaling. Current investigations suggest that it may have a role in pigmentation of melanocytes.

Presentation

Five slides of CITED1 positive tissues, each mounted on Hydrophilic Plus Slides, provided in a plastic mailer.

Catalog No.	Quantity
BSB-9446-CS	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 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. Shi G, et al. The transcriptional activity of CITED1 is regulated by phosphorylation in a cell cycle-dependent manner. J Biol Chem. 2006;281.37: 27426-27435.
2. Yahata T, et al. Selective coactivation of estrogen-dependent transcription by CITED1 CBP/p300-binding protein. Genes Dev. 2001;15.19: 2598-2612.
3. Shen X, et al. TGF- β -induced phosphorylation of Smad3 regulates its interaction with coactivator p300/CREB-binding protein. Mol Biol Cell. 1998;9.12: 3309-3319.
4. Yang D, et al. CBP/p300-interacting protein CITED1 modulates parathyroid hormone regulation of osteoblastic differentiation. Endocrinol. 2008;149.4: 1728-1735.
5. Dillon R, et al. An EGR2/CITED1 transcription factor complex and the 14-3-3 σ tumor suppressor are involved in regulating ErbB2 expression in a transgenic-mouse model of human breast cancer. Mol Cell Biol. 2007;27.24: 8648-8657.

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