

# Caspase-3 Control Slides



## Intended Use

For In Vitro Diagnostic Use.

## Summary and Explanation

Caspase-3 is a cysteine-aspartic acid protease encoded by the gene CASP3 on chromosome 4q35.1. Caspase-3 belongs to the caspase family, which is a family of enzymes crucial to mediating apoptosis. Once activated, Caspase-3 degrades intracellular proteins as well as functional proteins and induces cell death.

Dysregulated apoptosis is a typical characteristic of human cancer. Abnormal Caspase-3 expression has been directly associated with acute Myelogenous Leukemia, where Caspase-3 overexpression has been detected. On the contrary, decreased expression of Caspase-3 has been found in Prostate Cancer. Another study has found a significant association between high Caspase-3 levels and increased death rates in Breast Cancer using IHC, highlighting the prognostic potential of Caspase-3. In another study, IHC analysis of Gastric, Ovarian, Cervical, and Colorectal Cancer demonstrated that patients with high expression of cleaved Caspase-3 had a significantly shorter overall survival time compared with those with low cleaved Caspase-3 expression. Additionally, a link between Caspase-3 expression and tumor stage and lymph node metastasis was found in Gastric and Ovarian cancer. Caspase-3 is the predominant caspase involved in amyloid- $\beta$  precursor protein cleavage, consistent with its marked elevation in dying neurons of Alzheimer's disease brains and colocalization of its amyloid- $\beta$  precursor protein cleavage product with A $\beta$  in senile plaques. Caspases thus appear to play a dual role in proteolytic processing of amyloid- $\beta$  precursor protein and the resulting propensity for A $\beta$  peptide formation, as well as in the ultimate apoptotic death of neurons in Alzheimer's disease.

## Presentation

Five slides of Caspase-3 positive tissues, each mounted on Hydrophilic Plus Slides, provided in a plastic mailer.

| Catalog No. | Quantity |
|-------------|----------|
| BSB-9056-CS | 5 slides |
| BSB-3715-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 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<br>AP/HRP | PolyDetector<br>AP/HRP | PolyDetector<br>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

- Jiang M, Qi L, Li L, Li Y. The caspase-3/GSDME signal pathway as a switch between apoptosis and pyroptosis in cancer. Cell Death Discov. 2020;6:112. Published 2020 Oct 28. doi:10.1038/s41420-020-00349-02. Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. Cell Death Differ. 1999;6(2):99-104. doi:10.1038/sj.cdd.44004763. Pu X, Storr SJ, Zhang Y, et al. Caspase-3 and caspase-8 expression in breast cancer: caspase-3 is associated with survival. Apoptosis. 2017;22(3):357-368. doi:10.1007/s10495-016-1323-54. Hu Q, Peng J, Liu W, et al. Elevated cleaved caspase-3 is associated with shortened overall survival in several cancer types. Int J Clin Exp Pathol. 2014;7(8):5057-5070. Published 2014 Jul 15.5. National Center for Biotechnology Information. CASP3 caspase 3 [ Homo sapiens (human)]. Gene ID: 836, updated on 29-Nov-2020. <https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=836.6>. Gervais FG, Xu D, Robertson GS, et al. Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-beta precursor protein and amyloidogenic A beta peptide formation. Cell. 1999;97(3):395-406. doi:10.1016/s0092-8674(00)80748-57. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe WorkPractices 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 REP</b> | QAdvis EAR AB<br>Ideon Science Park<br>Scheelevägen 17<br>SE-223 70 Lund, Sweden                         |  Storage Temperature<br>Limites de température<br>Zulässiger Temperaturbereich                           |  Manufacturer<br>Fabricant<br>Hersteller              | <b>REF</b> | Catalog Number<br>Référence du catalogue<br>Bestellnummer |
| <b>IVD</b>    | In Vitro Diagnostic Medical Device<br>Dispositif médical de diagnostic in vitro<br>In-Vitro-Diagnostikum |  Read Instructions for Use<br>Consulter les instructions<br>d'utilisation<br>Gebrauchsanweisung beachten |  Expiration Date<br>Utiliser jusque<br>Verwendbar bis | <b>LOT</b> | Lot Number<br>Code du lot<br>Chargenbezeichnung           |