ZyBlack Quenching Solution

REF BS-0002-8 Σ 20 (8 ml)

For use in fluorescence *in situ* hybridization procedures

4250380S718N

CE IVD

In vitro diagnostic medical device

according to IVDR (EU) 2017/746

1. Intended use

The ZyBlack Quenching Solution (**BS2**) is intended to be used in fluorescence *in situ* hybridization (FISH) procedures to reduce autofluorescence on formalin-fixed, paraffin-embedded specimens. The ZyBlack Quenching Solution is intended to be used in combination with Zyto*Light* probes and the Zyto*Light* FISH-Tissue Implementation Kit (Prod. No. Z-2028-5/-20).

The product is intended for professional use only. All tests using the product should be performed in a certified, licensed anatomic pathology laboratory under the supervision of a pathologist/human geneticist by qualified personnel.

2. Test principle

The fluorescence *in situ* hybridization (FISH) technique allows for the detection and visualization of specific nucleic acid sequences in cell preparations. Fluorescently-labeled DNA fragments, so called FISH probes, and their complementary target DNA strands in the preparations are co-denatured and subsequently allowed to anneal during hybridization. Afterwards, unspecific and unbound probe fragments are removed by stringency washing steps. After counterstaining the DNA with DAPI, hybridized probe fragments are visualized using a fluorescence microscope equipped with excitation and emission filters specific for the fluorochromes with which the FISH probe fragments have been directly labeled.

3. Reagents provided

The ZyBlack Quenching Solution is available in one size:

- BS-0002-8: 8 ml (sufficient for 20 tests of 400 µl each)

4. Materials required but not provided

- Zyto*Light* probe
- Zyto*Light* FISH-Tissue Implementation Kit (Prod. No. Z-2028-5/-20)
- 25x Wash Buffer A (Prod. No.: WB-0002-50) or
- 5x F*lex*ISH Wash Buffer (Prod. No.: WB-0010-150/ -500)
- Deionized or distilled water

The ZyBlack Quenching Solution is intended to be used in ISH procedures using ZytoVision probes and kits. For information on materials required for ISH procedures, please refer to the instructions for use of the respective ZytoVision probe and implementation kit.

5. Storage and handling

Store at 2-8 °C in an upright position. Return to storage conditions immediately after use. Do not use reagents beyond expiry date indicated on the label. The product is stable until expiry date indicated on the label when handled accordingly.

6. Warnings and precautions

- Read the instructions for use prior to use!
- Do not use the reagents after the expiry date has been reached!
- This product contains substances (in low concentrations and volumes) that are harmful to health. Avoid any direct contact with the reagents. Take appropriate protective measures (use disposable gloves, protective glasses, and lab garments)!
- Report any serious incident that has occurred in relation to the product to the manufacturer and the competent authority according to local regulations!
- If reagents come into contact with skin, rinse skin immediately with copious amounts of water!
- A material safety data sheet is available on request for the professional user.
- Do not reuse reagents, unless reuse is explicitly permitted!
- Avoid cross-contamination of samples as this may lead to erroneous results.
- The specimens must not be allowed to dry during the hybridization and washing steps.

Hazard and precautionary statements:

This product is not classified as hazardous according to Regulation (EC) No. 1272/2008.

7. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- For non-automated use only.
- The clinical interpretation of any positive staining, or its absence, must be done within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. It is the responsibility of a qualified pathologist/human geneticist to be familiar with the ISH probes, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist/human geneticist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
- Specimen staining, especially signal intensity and background staining, is dependent on the handling and processing of the specimen prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other specimens or fluids may produce artefacts or false results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the specimen.
- The performance was validated using the procedures described in the instruction for use of the respective ZytoVision probe and implementation kit. Modifications to these procedures might alter the performance and have to be validated by the user. This IVD is only certified as CE when used as described in this instruction for use within the scope of the intended use.

8. Interfering substances

Refer to the instructions for use of the respective ZytoVision probe and implementation kit.

9. Preparation of specimens

Refer to the instructions for use of the respective ZytoVision probe and implementation kit. Treatment with organic solvents such as ethanol on day 1 after ZyBlack application and before hybridization will remove the ZyBlack staining effect and thus no reduction of autofluorescence will be visible.

10. Preparatory treatment of the device

The device is ready-to-use. No reconstitution, mixing, or dilution is required.

11. Assay procedure

The ZyBlack Quenching Solution (**BS2**) can be easily incorporated into FISH protocols from ZytoVision GmbH by applying it after the proteolytic pretreatment of formalin-fixed, paraffin-embedded specimens (for detailed information on how to perform FISH with ZytoVision products, please refer to the instruction for use of the respective Zyto*Light* probe and kit).

(1) Bring ZyBlack Quenching Solution (**BS2**) to room temperature before use.

(2) Complete the proteolytic pretreatment:
- Wash 1x 5 min at room temperature in Wash Buffer SSC (**WB1**)
- Wash 1x 1 min at room temperature in deionized water.
- Dehydration: in 70%, 90%, and 100% ethanol, each for 1 min.
- Air dry sections completely.

(3) Apply an appropriate amount of ZyBlack Quenching Solution (**BS2**) on the specimen.

(4) Incubate for 30 min at room temperature on a flat surface.

(5) Wash 2x 5 min at room temperature in 1x Wash Buffer A (**WB2**) or 1x F*ex*ISH Wash Buffer (**WB10**) (prepared as described in the instructions for use of the respective buffer).

(6) Wash 1x 1 min in deionized water.

(7) Air-dry specimens for at least 30 min.

(8) Proceed with hybridization of the ZytoVision probe.

Optional, when performing post-fixation step:
Complete the post-fixation before using the ZyBlack Quenching Solution.

12. Interpretation of results

Refer to the instructions for use of the respective ZytoVision probe.

13. Recommended quality control procedures

Refer to the instructions for use of the respective ZytoVision probe.

14. Performance characteristics

Refer to the instructions for use of the respective ZytoVision probe.

15. Disposal

The disposal of reagents must be carried out in accordance with local regulations.

16. Troubleshooting

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all. Please refer to the instructions for use of the respective ZytoVision probe and kit for further information.

17. Literature

- Kievits T, et al. (1990) *Cytogenet Cell Genet* 53: 134-6.
- Wilkinson DG: In Situ Hybridization, A Practical Approach, *Oxford University Press* (1992) ISBN 0 19 963327 4.

18. Revision

www.zytovision.com

Please refer to www.zytovision.com for the most recent instructions for use as well as for instructions for use in different languages.

Our experts are available to answer your questions.
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