ZipFISH™ Hybridization Buffer

Diluent for fluorescent *in situ* hybridization probes
901-7323-102517

BIOCARE MEDICAL

Catalog Number: **FRI7323 P, C**

Description: 0.2, 1.0 ml, Ready-to-use

Intended Use:
For In Vitro Diagnostic Use
ZipFISH Hybridization Buffer is a buffer used to dilute DNA probes for use in Fluorescence *in situ* Hybridization (FISH) procedures. The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
ZipFISH Hybridization Buffer is a buffer that accelerates the hybridization of FISH DNA probes to their complementary target sequence on the test tissue. Effective hybridization facilitates enhanced staining and reduces artifacts.

Known Applications:
Fluorescence *in situ* hybridization on formalin-fixed paraffin-embedded (FFPE) tissues

Supplied As:
Proprietary combination of formamide, buffers and other staining enhancers

Materials and Reagents Needed But Not Provided:
Positively charged microscope slides
Desert Chamber* (drying oven)
Positive and negative tissue controls
Xylene (Could be replaced with a xylene substitute*)
Ethanol or reagent alcohol
Water bath
Deionized or distilled water
Pretreatment reagent
2X SSC Buffer
0.2N HCl
Protease buffer and pepsin
Slide denaturation / hybridization equipment
Fluorescence labeled DNA probes*
Negative control reagents*
SSC Wash Buffer*
Mounting media*
Nuclear counterstain

*Biocare Medical Products: Refer to a Biocare Medical catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.

Storage and Stability:
Store ZipFISH Hybridization Buffer at -20ºC and away from light. The product is stable to the expiration date printed on the label when stored under these conditions. Do not use after expiration date.

Instructions for Use:
ZipFISH Hybridization Buffer is provided in a ready-to-use format. Fluorescent probes can be diluted to the desired working concentration in this buffer (suggested 5X of current). We recommend thawing the buffer at room temperature (RT) 1 hour prior to use, and then mixing vigorously by repeatedly tapping, inverting, shaking and pipetting up and down at least 10 times. Dilute FISH probe in ZipFISH Hybridization Buffer and pipette up and down several times to mix well. Empirical testing will determine the optimal dilution and best performance. Follow your standard FISH procedure with a hybridization at 37°C for 1-2 hours.

Instructions for Use Cont'd:

Protocol Recommendations for FISH on FFPE tissues:
Preheat slides in 60°C oven for 30 minutes before dewax.

Deparaffinization
1. Dewax slides in Xylene-substitute x 3 for 5 minutes each at RT.
2. 100% Ethanol washes x 2 for 3 minutes each at RT.
3. 85% and 70% Ethanol for 3 minutes each at RT.
4. Deionized water for 2 minutes at RT.

Retrieval / Protein Digestion
5. 0.2N HCl at RT for 20 minutes.
6. Prewarmed pretreatment reagent at 80°C for 40 minutes.
7. 2X SSC Buffer at RT for 2 minutes.
8. Deionized water at RT for 2 minutes.
9. 0.2N HCl at RT for 2 minutes.
10. Prewarmed 50 ml of protease buffer solution (add 20mg of pepsin 15 min prior to use) for 35 minutes at 37°C.
11. Deionized water for 2 minutes at RT.
12. Dehydration: 70%, 85% and 100% for 2 minutes each at RT.
13. Air dry slides.

Probe Hybridization
14. Mix FISH probe with ZipFISH Hybridization Buffer (see instructions for use).
15. Apply probe: 10µL and cover with 18x18 coverslip; no need to seal with rubber cement.
16. Load slides onto denaturation / hybridization instrument: denature at 83°C for 5 minutes, then hybridize at 37°C for 1-2 hours.

Post-Hybridization Washing
17. Remove slides from denaturation / hybridization instrument. Place into SSC Wash Buffer at RT, agitate gently for about 5 minutes until coverslips fall off. Gently shake slides for an additional minute.
18. Place slides into 72°C±1ºC SSC Wash Buffer. Incubate for 2 minutes without shaking.
19. Carefully transfer slides to room temperature SSC Wash Buffer. Incubate for 2 minutes.

Counterstaining
20. Apply 10µl of a DAPI nuclear counterstain (1µg/ml) directly to the target area of the slide; wash with deionized water, then apply fluorescent mounting media; cover area using a 24 mm x 50 mm cover glass.
21. Slides are ready for visualization using a fluorescent microscope.

Limitations:
This reagent has been optimized for use with Biocare's FISH probes. The optimum probe dilution and protocols for specific applications may vary. These include, but are not limited to; sample preparation, hybridization conditions and incubation times, post hybridization washes and microscope filter specifications and illumination conditions. Third party FISH probes may be diluted in this buffer; however, appropriate probe concentration and protocol parameters may depend upon multiple factors and must be empirically determined by the user. The recommended hybridization times and wash conditions are for guidance only and it is the responsibility of the operator to determine

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Limitations Cont'd:
optimal conditions. The clinical interpretation of any positive or negative hybridization events should be evaluated within the context of clinical presentation, morphological and/or cytogenetic criteria by a suitably qualified practitioner. These findings should be complemented by the use of appropriate controls and other diagnostic tests where appropriate.

Quality Control:
Fluorescence In-Situ Hybridization (FISH) Methods for Clinical Laboratories; Approved Guidelines – Second edition (MM07-A2). CLSI, Wayne, PA (www.clsi.org). 2013.

Precautions:
1. This product is intended for in vitro diagnostic (IVD) use.
2. This product contains formamide, which may be toxic. Formamide and other staining enhancers may cause serious eye damage or reproductive toxicity. These chemicals may also cause irritation by inhalation or skin contact. Avoid any direct contact exposure to reagent. Take appropriate protective measures (use disposable gloves, protective glasses and lab garments).
3. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Avoid contacting the skin and mucous membranes with reagents and specimens, and follow standard laboratory precautions to prevent exposure to eyes and skin. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.
4. Microbial contamination of reagents may result in an increase in nonspecific staining.
5. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:
Follow the reagent specific protocol recommendations according to the data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:
1. Wilkinson DG. In Situ Hybridization: A Practical Approach (Practical Approach Series). 2nd Ed. Oxford: Oxford University Press, 1999.
2. Nuovo GJ. In Situ Molecular Pathology and Co-Expression Analyses. 1st Ed. San Diego: Academic Press, 2013.
3. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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