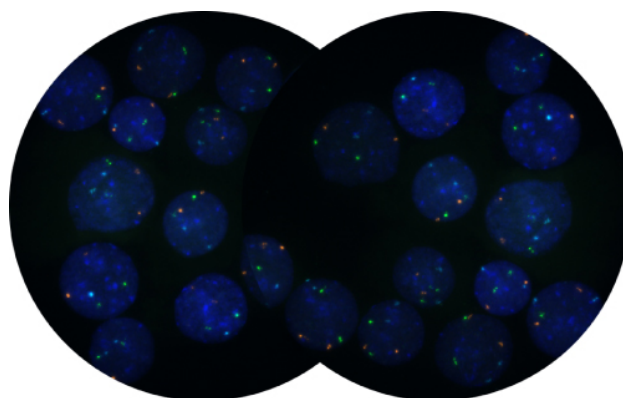




CytoTest DNA FISH Probe Instructions for Use



Applicable to following REF groups
CT-PAC
CT-LSP
CT-CCP

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PRODUCT INFORMATION

Key to Symbols



Catalog number



Name and address of Manufacturer



In vitro diagnostic medical device



Authorized representative in the European Community



Contains sufficient reagent for < n > tests



Biological risks



Batch number



Consult instructions for use



Use by YYYY-MM-DD



Minimum/maximum temperature



Non sterile

Intended User

CytoTest FISH Probes are intended for **Professional Use Only**.

Product Common Name

DNA Fluorescence *in situ* hybridization (FISH) probes

Intended Use

The FISH Probe is intended for use in an assay detecting known or suspected cytogenetic or chromosomal abnormality.

Indications for Use

The initial assessment of many hematologic and other malignancies typically includes morphological, histological, immunophenotypic (flow cytometric and/or immunohistochemical), and conventional cytogenetic analyses. FISH analysis can be an integral component of the diagnostics evaluation for any diseases involving genomic aberrations, in addition to or following conventional karyotyping, but particularly in cases of:

1. Abnormalities with a low frequency, but well-documented percentage, of false-negative cytogenetic results, particularly in scenarios where the clinical, hematologic, and pathological parameters suggest a specific abnormality
2. Abnormalities with a high frequency of "false-negative" cytogenetics
3. Interphase analysis, when conventional cytogenetics fails or is not possible, for example, on fixed

- tissue
4. To clarify abnormal or complex conventional karyotypic findings; and
 5. As a surrogate marker for a primary genetic event

Contraindications

The device is subject to the following limitations:

1. This product is not intended for high-risk uses such as therapy selection, therapeutic response prediction or screening for disease. The use of this device for assessing risk, disease monitoring, diagnosis and prognosis has not been established.
2. Clinical, pathology and other relevant information should always be correlated with FISH test results on patient samples. Patient clinical status should be taken into consideration when performing the test and utilizing the results.
3. The FISH test is not appropriate to determine the following abnormalities:
 - a. One or more point mutations in target DNA, or
 - b. Any other single nucleotide-level aberrations,
 - c. Small (below kb-range) deletions, insertions, inversions
 - d. Breakpoint identification at base pair level accuracy
4. This test does not allow determination of gene expression level or transcript type, and does not include measurement of gene product amount or integrity.
5. Interpretation of results: Unusual spot/signal patterns may be observed in rare cases. Such unexpected patterns may have unknown significance and may not be interpretable by this test alone. Metaphase analysis may be helpful in the characterization of some atypical or unexpected signal patterns.
6. FISH probe performance characteristics have been validated on samples of human peripheral blood lymphocytes and tissue samples.
7. Use of device for testing other sample types may have not been extensively tested and may require protocol optimization and adjustment.

Procedure Principles

Fluorescence *In situ* hybridization (FISH) is a powerful technique designed to detect presence or absence, location, integrity and amount of DNA or RNA sequences in tissues, cells or on chromosomes. FISH is based on the detection of specific sequences by pairing of bases (hybridization) on complementary single strands of nucleic acid. Here, one of the strands is a fluorescently labeled sequence fragment (probe) that binds only to those parts of the genome with sequences highly or completely complementary to the probe sequence, and the other strand is present in the sample material that is to be analyzed. Accordingly, *in situ* hybridization starts with preparing the sample to be analyzed and with preparing the probe. The typically double-stranded DNA in the sample has to be melted (denatured) into single strands, and the probe has to be fluorescently labeled to enable detection.

Product Description

CytoTest FISH probes are *in vitro* diagnostic medical devices and manufactured with genomic DNA obtained either from microdissected human chromosomes or cloned DNA fragments, depending on the probe type.

For optimal results, microscope filter sets, which are compatible with the fluorescence of the probes, must be selected.

Fluorophore	Excitation Peak (nm)	Emission Peak (nm)	Compatibility with Other Dyes
CytoRed™	583	605	SpectrumRed Propidium iodide (543-614)
CytoOrange™	551	575	SpectrumOrange

CytoGold™	523	549	SpectrumGold
CytoGreen™	495	518	SpectrumGreen Fluorescein isothiocyanate (FITC)
CytoAqua™	422	471	SpectrumAqua
CytoBlue™	402	421	SpectrumBlue (400-450)

Warnings and Precautions

Excessive exposure to light can photobleach the probe's fluorophores. Please take appropriate precautions when handling all reagents and slides containing the probe to avoid direct and prolonged light exposure. It is recommended to comply with the instructions described in this Instruction for Use when handling and using CytoTest FISH probes.

Experiment operators should wear suitable protective clothing, gloves and eye/face protection. Reagents used in FISH experiment may irritate eyes and skin; avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Inappropriate handling during transportation or storage can potentially degrade or impair the performance of the product. Any compromised products should be discarded according to any applicable law or regulations in your institution, region and/or country, and the reagents should not be used in any tests. If you have any concerns about degradation in the product's quality or performance, please contact the manufacturer or your regional distributor(s).

Storage and Handling

CytoTest FISH probes should be stored at -15°C to -25°C and protected from light. Avoid repeated freeze/thaw cycles. Please check the expiration date on the product label before use. These storage and handling conditions apply to both opened and unopened products.

Materials Provided

CytoTest DNA FISH probes provided in ready-to-use concentration.

Laboratory Equipment Required but Not Provided

- Microliter pipettor (1 to 10 µL) and clean tips
- Polypropylene microcentrifuge tubes (0.5 mL or 1.5 mL)
- 22 mm x 22 mm glass coverslips
- Rubber cement
- Graduated cylinder
- Diamond-tipped scribe
- Timer
- Forceps
- Coplin jars
- Media bottles (250 mL)
- Calibrated thermometer
- Vortex mixer
- Microcentrifuge
- Water baths (37 ± 2°C, 72 ± 2°C, and 80 ± 2°C)
- Air incubator (37 ± 2°C)
- Slide warmer
- Phase contrast light microscope
- Fluorescent microscope equipped with recommended filters

ASSAY PROCEDURE

(The experimental conditions in this user manual are general recommendations and are subject to change, depending on the condition of the sample material. They may require adjustment for certain sample types.)

FISH Procedure for FFPE Specimens

Reagents Required but Not Provided

- Paraffin Pretreatment Reagent Kit (Cat No: CT-ACC112-05):
 - Pretreatment Solution (50 ml): store at room temperature (RT)
 - Protease Buffer (62.5 ml, pH 2.0): store at RT
 - Protease (250 mg): Lyophilized, store at -20°C
- FISH Reagent Kit (Cat No: CT-ACC101-20):
 - 20X Sodium Chloride-Sodium Citrate Buffer (SSC) Salt: store at RT, avoid humidity
 - 4',6-diamidino-2-phenylindole (DAPI) Counterstain: store at 4°C in the dark
 - NP-40 (octylphenoxypolyethoxyethanol, or Nonidet P-40): store at RT
- Xylene: store at RT
- Ethanol (100%): store at RT
- Purified water: store at room RT
- Concentrated (12N) HCl: store at room RT

Preparation of Working Solutions

1. 20X SSC Solution (pH 7.0)

Reagents	Amount added	Final Concentration
SSC Salt	66 g	20X
Deionized H ₂ O (dH ₂ O)	250 ml	
TOTAL	250 ml	

2. Protease Solution

Reagents	Amount added	Final Concentration
Protease, lyophilized	250 mg	4 mg/ml
Protease Buffer	62.5 ml	
TOTAL	62.5 ml	

3. 90% Ethanol

Reagents	Amount added	Final Concentration
Ethanol (100%)	90 ml	90%
dH ₂ O	10 ml	
TOTAL	100 ml	

4. 70% Ethanol

Reagents	Amount added	Final Concentration
Ethanol (100%)	70 ml	70%
dH ₂ O	30 ml	
TOTAL	100 ml	

5. Post-hybridization Wash Solution (pH 7.0)

Reagents	Amount added	Final Concentration
20X SSC Solution	10 ml	2X
NP-40	300 µl	0.3%
dH ₂ O	90 ml	
TOTAL	100 ml	

FISH Procedure for Paraffin-embedded Tissue Sections

Slide Pretreatment

1. Immerse slides in xylene at RT for 10 minutes. Repeat twice with fresh xylene each time.
2. Dehydrate slides in 100% ethanol at RT for 5 minute. Repeat once with fresh 100% ethanol.
3. Air dry slides for 2-5 minutes, if desired.
4. Immerse slides in pre-warmed Pretreatment Solution at 80°C for 10 minutes.
5. Immerse slides in purified water at RT for 3 minute.

Protease Pretreatment

1. Immerse slides in Protease Solution at 37°C for 10-60 minutes (depending on the condition of samples) and monitor the condition of cells under a light microscope.
2. Immerse slides in purified water at RT for 3 minutes.
3. Air dry slides for 2-5 minutes.

Slide Dehydration

1. Immerse slides in 70% ethanol for 3 minutes.
2. Immerse slides in 90% ethanol for 3 minutes.
3. Immerse slides in 100% ethanol for 3 minutes.
4. Air dry slides.

Probe Preparation

1. Pre-warm the probe at RT for 20-30 minutes.
2. Briefly vortex and spin down the probe.

Co-denaturation & Hybridization

1. Apply 10 µl of the probe on each hybridization area and cover with a 22 mm x 22 mm coverslip. Seal coverslip(s) with rubber cement.
2. Co-denature slides with probe at 72°C for 5 minutes.
3. Place slides in a pre-warmed humidified hybridization chamber and incubate slides at 37°C overnight (16 hours).

Post-hybridization Wash

1. Mark each hybridization area on the back of the slides with a diamond-tip pen.
2. Carefully remove rubber cement.
3. Immerse slides in Post-hybridization Wash Solution at RT to loosen the coverslips. Shake gently to allow the coverslips to detach unaided; do not pull the coverslips off.
4. Immerse slides in pre-warmed Post-hybridization Wash Solution at 72°C for 2 minutes.

Slide Dehydration

1. Immerse slides in 70% ethanol for 2 minutes.
2. Immerse slides in 90% ethanol for 2 minutes.
3. Immerse slides in 100% ethanol for 2 minutes.
4. Air dry slides in the dark.

Visualization

1. Apply DAPI counterstain and cover slides with coverslips.
2. Examine slides under a fluorescence microscope with proper filter sets.

FISH Procedure for Cytological Specimens

Reagents Required but Not Provided

- FISH Reagent Kit (Cat No: CT-ACC101-20):
 - 20X SSC Salt: store at RT, avoid humidity
 - DAPI Counterstain: store at 4°C in the dark
 - NP-40: store at RT
- Pepsin (Lyophilized): store at -20°C or below
- Hydrochloric acid (1N): store at RT
- Formaldehyde (37%): store at RT

- 10X Phosphate-buffered Saline (PBS) Solution: store at RT
- Ethanol (100%): store at RT

Preparation of Working Solutions

1. 20X SSC Solution (pH 7.0)

Reagents	Amount added	Final Concentration
SSC Salt	66 g	20X
dH ₂ O	250 ml	
TOTAL	250 ml	

2. Pepsin Stock Solution

Reagents	Amount added	Final Concentration
Pepsin, lyophilized	100 mg	100 mg/ml
dH ₂ O	1 ml	
TOTAL	1 ml	

Note: Keep on ice. Make 20 µl aliquots, store at -20°C.

3. Pepsin Working Solution

Reagents	Amount added	Final Concentration
HCl (1N)	1 ml	0.01N
Pepsin Stock Solution	20 µl	0.02 µg/µl
dH ₂ O	99 ml	
TOTAL	100 ml	

4. 2X SSC Solution

Reagents	Amount added	Final Concentration
20X SSC Solution	10 ml	2X
dH ₂ O	90 ml	
TOTAL	100 ml	

5. 1X PBS Solution

Reagents	Amount added	Final Concentration
10X PBS Solution	10 ml	1X
dH ₂ O	90 ml	
TOTAL	100 ml	

6. Formaldehyde Solution

Reagents	Amount added	Final Concentration
Formaldehyde (37%)	2.7 ml	1%
10X PBS Solution	10 ml	1X
dH ₂ O	89 ml	
TOTAL	100 ml	

7. 90% Ethanol

Reagents	Amount added	Final Concentration
Ethanol (100%)	90 ml	90%

dH ₂ O	10 ml
TOTAL	100 ml

8. 70% Ethanol		
Reagents	Amount added	Final Concentration
Ethanol (100%)	70 ml	70%
dH ₂ O	30 ml	
TOTAL	100 ml	

9. Post-hybridization Wash Solution 1		
Reagents	Amount added	Final Concentration
20X SSC Solution	2 ml	0.4X
NP-40	300 µl	0.3%
dH ₂ O	98 ml	
TOTAL	100 ml	

10. Post-hybridization Wash Solution 2		
Reagents	Amount added	Final Concentration
20X SSC Solution	10 ml	2X
NP-40	100 µl	0.1%
dH ₂ O	90 ml	
TOTAL	100 ml	

FISH Procedure for Cytology

Slide Preparation

1. Equilibrate slides in 2X SSC Solution at RT for 2 minutes.
2. Immerse slides in pre-warmed Pepsin Working Solution at 37°C for 1-10 minutes (depending on the condition of samples) and monitor the condition of cells under a light microscope.
3. Wash slides in 1X PBS Solution at RT for 5 minutes.
4. Post-fix slides in Formaldehyde Solution at RT for 5 minutes.
5. Wash slides in 1X PBS Solution at RT for 5 minutes.

Slide Dehydration

1. Immerse slides in 70% ethanol for 3 minutes.
2. Immerse slides in 90% ethanol for 3 minutes.
3. Immerse slides in 100% ethanol for 3 minutes.
4. Air dry slides.

Probe Preparation

1. Pre-warm probes at RT for 20~30 minutes.
2. Briefly vortex and spin down probes.

Co-denaturation & Hybridization

1. Apply 10 µl of the probe on each hybridization area and cover with a 22 mm x 22 mm coverslip. Seal coverslip(s) with rubber cement.
2. Co-denature slides with probe at 72°C for 2 minutes.
3. Place slides in a pre-warmed humidified hybridization chamber and incubate slides at 37°C overnight (16 hours).

Post-hybridization Wash

1. Mark hybridization area on the back of the slides with a diamond-tip pen.
2. Carefully remove the rubber cement.
3. Soak slide in 2X SSC Solution at RT to loosen coverslip(s). Do not pull off coverslip(s).
4. Immerse slides in pre-warmed Post-hybridization Wash Solution 1 at 72°C for 1 minute.
5. Immerse slides in Post-hybridization Wash Solution 2 at RT for 2 minutes.
6. Air dry slides.

Visualization

1. Apply DAPI counterstain, cover with coverslip(s).
2. Examine slides under a fluorescence microscope with proper filter sets.

FISH Procedure for Amniotic Fluid Specimens

Reagents Required but Not Provided

- FISH Reagent Kit:
 - 20X SSC Salt: store at RT, avoid humidity
 - DAPI Counterstain: store at 4°C in the dark
 - NP-40: store at RT
- Pepsin (Lyophilized): store at -20°C or below
- Hydrochloric acid (1N): store at RT
- Formaldehyde (37%): store at RT
- 10X PBS Solution: store at RT
- Ethanol (100%): store at RT

Preparation of Working Solutions

1. 20X SSC Solution (pH 7.0)

Reagents	Amount added	Final Concentration
SSC Salt	66 g	20X
dH ₂ O	250 ml	
TOTAL	250 ml	

2. Pepsin Stock Solution

Reagents	Amount added	Final Concentration
Pepsin, lyophilized	100 mg	100 mg/ml
dH ₂ O	1 ml	
TOTAL	1 ml	

Note: Keep on ice. Make 20 µl aliquots, store at -20°C.

3. Pepsin Working Solution 1 (for uncultured specimens)

Reagents	Amount added	Final Concentration
HCl (1N)	1 ml	0.01N
Pepsin Stock Solution	50 µl	0.05 µg/µl
dH ₂ O	99 ml	
TOTAL	100 ml	

4. Pepsin Working Solution 2 (for cultured specimens)

Reagents	Amount added	Final Concentration
HCl (1N)	1 ml	0.01N
Pepsin Stock Solution	20 µl	0.02 µg/µl

dH ₂ O	99 ml
TOTAL	100 ml

5. 2X SSC Solution

Reagents	Amount added	Final Concentration
20X SSC Solution	10 ml	2X
dH ₂ O	90 ml	
TOTAL	100 ml	

6. 1X PBS Solution

Reagents	Amount added	Final Concentration
10X PBS Solution	10 ml	1X
dH ₂ O	90 ml	
TOTAL	100 ml	

7. Formaldehyde Solution

Reagents	Amount added	Final Concentration
Formaldehyde (37%)	2.7 ml	1%
10X PBS Solution	10 ml	1X
dH ₂ O	89 ml	
TOTAL	100 ml	

8. 90% Ethanol

Reagents	Amount added	Final Concentration
Ethanol (100%)	90 ml	90%
dH ₂ O	10 ml	
TOTAL	100 ml	

9. 70% Ethanol

Reagents	Amount added	Final Concentration
Ethanol (100%)	70 ml	70%
dH ₂ O	30 ml	
TOTAL	100 ml	

10. Post-hybridization Wash Solution 1

Reagents	Amount added	Final Concentration
20X SSC Solution	2 ml	0.4X
NP-40	300 µl	0.3%
dH ₂ O	98 ml	
TOTAL	100 ml	

11. Post-hybridization Wash Solution 2

Reagents	Amount added	Final Concentration
20X SSC Solution	10 ml	2X
NP-40	100 µl	0.1%

dH ₂ O	90 ml
TOTAL	100 ml

FISH Procedure for Amniocytes

Slide Preparation

Uncultured Specimens:

1. Equilibrate slides in 2X SSC Solution at 37°C for 1 hour.
2. Immerse slides in pre-warmed Pepsin Working Solution 1 at 37°C for 1-10 minutes (depending on the condition of samples) and monitor the condition of cells under a light microscope.
3. Wash slides in 1X PBS Solution at RT for 5 minutes.
4. Post-fix slides in Formaldehyde Solution at RT for 5 minutes.
5. Wash slides in 1X PBS Solution at RT for 5 minutes.

Cultured Specimens:

1. Equilibrate slides in 2X SSC Solution at RT for 2 minutes.
2. Immerse slides in pre-warmed Pepsin Working Solution 2 at 37°C for 1-10 minutes (depending on the condition of samples) and monitor the condition of cells under a light microscope.
3. Wash slides in 1X PBS Solution at RT for 5 minutes.
4. Post-fix slides in Formaldehyde Solution at RT for 5 minutes.
5. Wash slides in 1X PBS Solution at RT for 5 minutes.

Slide Dehydration

1. Immerse slides in 70% ethanol for 3 minutes.
2. Immerse slides in 90% ethanol for 3 minutes.
3. Immerse slides in 100% ethanol for 3 minutes.
4. Air dry slides.

Probe Preparation

1. Pre-warm probes at RT for 20~30 minutes.
2. Briefly vortex and spin down probes.

Co-denaturation & Hybridization

1. Apply 10 µl of the probe on each hybridization area and cover with a 22 mm x 22 mm coverslip. Seal coverslip(s) with rubber cement.
2. Co-denature slides with probe at 72°C for 2 minutes.
3. Place slides in a pre-warmed humidified hybridization chamber and incubate slides at 37°C overnight (16 hours).

Post-hybridization Wash

1. Mark hybridization areas on the back of the slides with a diamond-tip pen.
2. Carefully remove the rubber cement.
3. Soak slide in 2X SSC Solution at RT to loosen coverslip(s). Do not pull off coverslip(s).
4. Immerse slides in pre-warmed Post-hybridization Wash Solution 1 at 72°C for 1 minute.
5. Immerse slides in Post-hybridization Wash Solution 2 at RT for 2 minutes.
6. Air dry slides.

Visualization

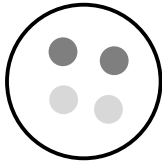
1. Apply DAPI counterstain, cover with coverslip(s).
2. Examine slides under a fluorescence microscope with proper filter sets.

INTERPRETATION OF RESULTS

Signal Patterns for Amplification/Deletion Probes

Normal Patterns

- 2 orange signals + 2 green signals (2O2G)



Color Key

- Orange signals represented by dark grey dots
- Green signals represented by light grey dots

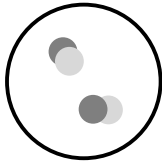
Abnormal Patterns

- Any other patterns

Signal Patterns for Break-apart Probes

Normal Patterns

- 2 Orange-green overlapped signals (2OG)



Color Key

- Orange signals represented by dark grey dots
- Green signals represented by light grey dots
- Overlapping orange and green signals can appear as yellow.

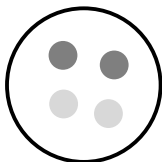
Abnormal Patterns

- Any other patterns

Signal Patterns for Fusion/Translocation Probes

Normal Patterns

- 2 orange signals + 2 green signals (2O2G)



Color Key

- Orange signals represented by dark grey dots
- Green signals represented by light grey dots

Abnormal Patterns

- Any other patterns. Fused signals (overlapping of orange and greens signals) can appear as yellow signals.

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