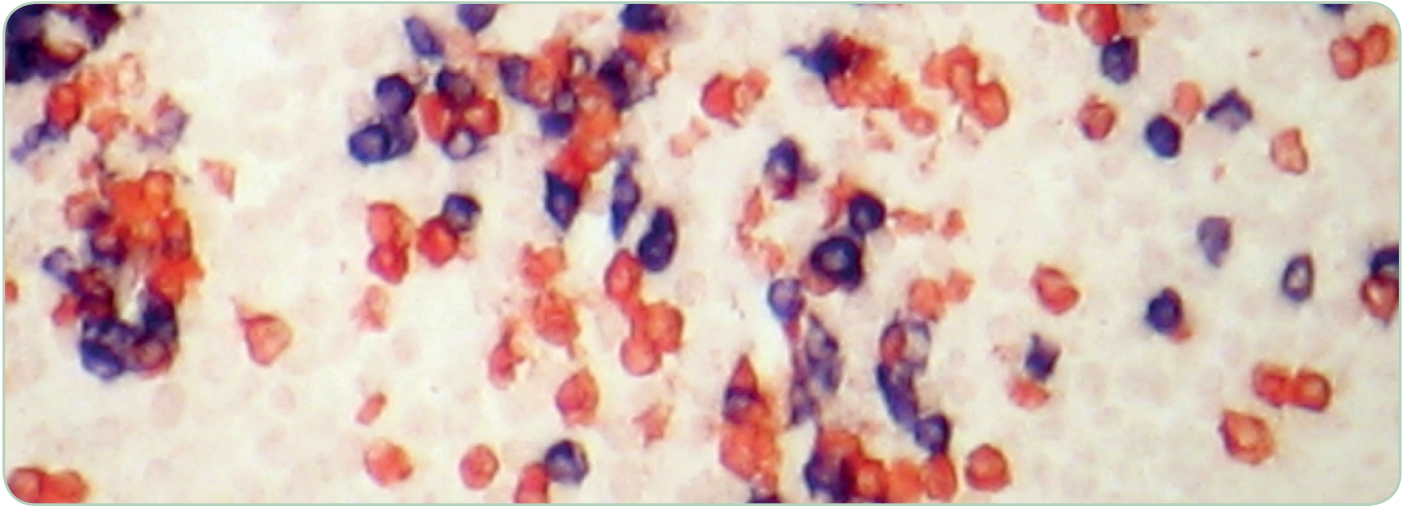


## Achieving Chromogenic *in situ* Hybridization Results in just 4 Hours!



### Introduction

The ZytoFast® products are designed for outstandingly fast detection and discrimination of human pathogen viruses, e.g. HPV, EBV, CMV, and the determination of lymphocyte clonality by detecting Ig-κ and Ig-λ light chain RNA by Chromogenic *in situ* Hybridization (CISH) in formalin-fixed, paraffin-embedded tissue sections and cell samples.

### ZytoFast®: Outstandingly fast CISH

Optimized protocols and faster tissue penetration due to short oligonucleotide probes of the ZytoFast® system, make the ZytoFast® CISH procedure outstandingly fast.

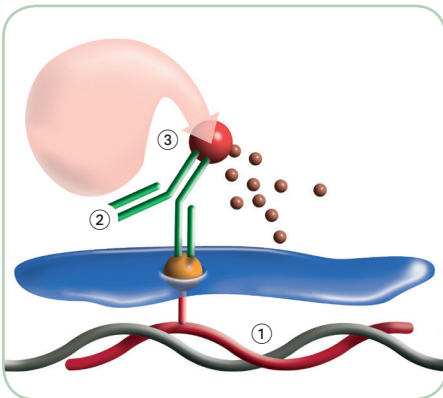
Single color results can be achieved within just 4 hours, hands-on time is about 2 hours!

### High Sensitivity and Specificity

All ZytoFast® probes are tagged using the unique ZytoFast® HighTag System providing improved signal intensity! High specificity without risk of cross-hybridizations is obtained due to optimized oligonucleotide probes.

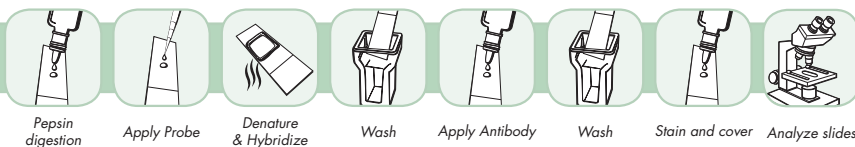
### Advantages of CISH

- Simultaneous observation of tissue morphology and CISH signals
- No risk of false positives due to mispriming or contamination as with PCR
- Easy method comparable to IHC
- No costly equipment needed
- Ability to test archival specimens
- High sensitivity and specificity

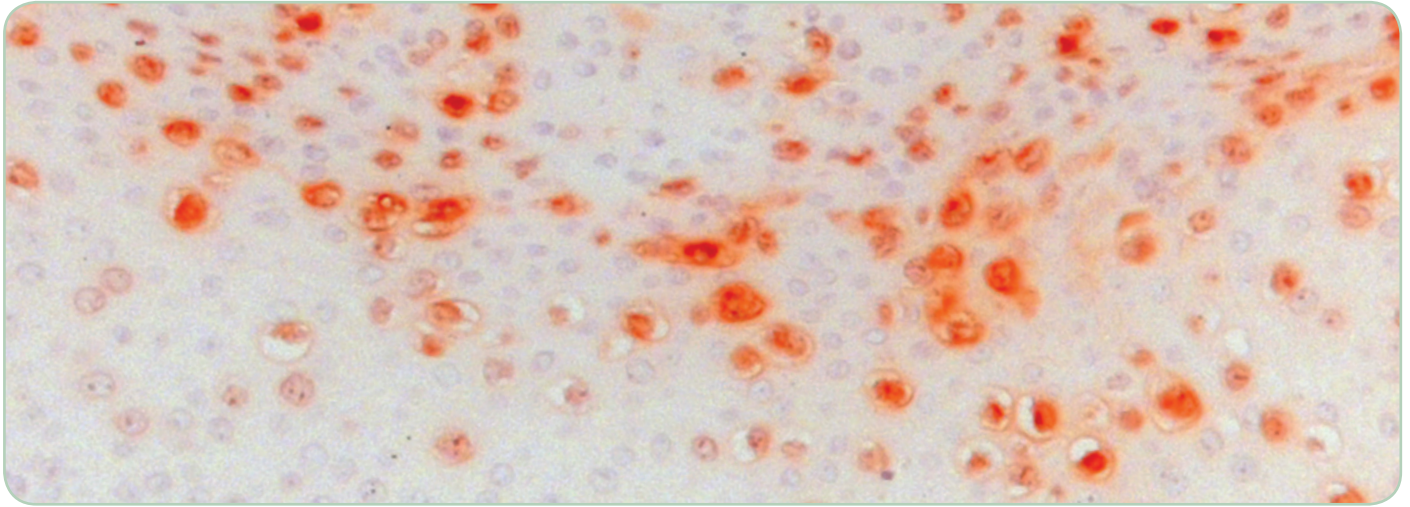


The ZytoFast® system uses oligonucleotide probes tagged with Biotin or Digoxigenin (1) which are detected using enzyme-conjugated antibodies or streptavidin targeting the tags (2). The enzymatic reaction of chromogenic substrates (3), e.g. BCIP/NBT or AEC, leads to the formation of strong color precipitates that can be visualized by light microscopy.

### Protocol Overview



## ZytoFast<sup>®</sup> PLUS for Increased Sensitivity!



### Introduction

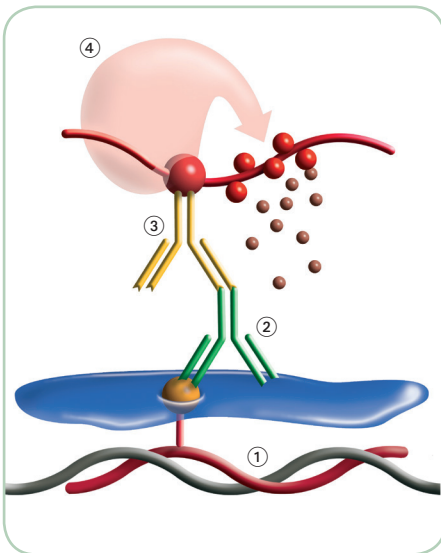
The ZytoFast<sup>®</sup> PLUS products are designed for outstandingly fast and sensitive detection and discrimination of human pathogen viruses, e.g. HPV, EBV, CMV, and the determination of lymphocyte clonality by detecting Ig-κ and Ig-λ light chain RNA by Chromogenic *in situ* Hybridization (CISH) in formalin-fixed, paraffin-embedded tissue sections and cell samples. The signal intensity of ZytoFast<sup>®</sup> probes is increased even more when using the ZytoFast<sup>®</sup> PLUS Implementation Kits.

### ZytoFast<sup>®</sup> PLUS – Outstandingly fast and sensitive CISH

Depending on the time required for dewaxing and pretreatment of tissue sections, ZytoFast<sup>®</sup> PLUS protocols can be performed within approx. 4 hours! Thus, due to optimized protocols, the ZytoFast<sup>®</sup> PLUS method takes only slightly more time compared to ZytoFast<sup>®</sup> protocols while being much more sensitive!

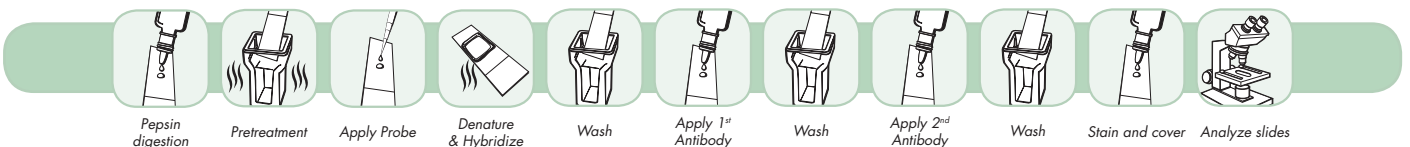
### ZytoFast<sup>®</sup> PLUS – Flexibility that meets your Needs

Several ZytoFast<sup>®</sup> PLUS CISH Implementation Kits using different enzyme/substrate combinations can be combined with any separately available Digoxigenin-labeled ZytoFast<sup>®</sup> probe to meet your preferences concerning the detection chemistry, counterstaining, and embedding. Each ZytoFast<sup>®</sup> PLUS CISH Implementation Kit includes a detailed protocol, all necessary reagents as well as positive and negative control probes for versatile use in DNA as well as RNA *in situ* hybridizations.



The ZytoFast<sup>®</sup> PLUS system uses Digoxigenin-labeled probes ① which are detected using primary antibodies ②. These antibodies are detected by polymerized enzyme-conjugated secondary antibodies ③. The enzymatic reaction of chromogenic substrates ④, e.g. NBT/BCIP or DAB, leads to the formation of strong color precipitates that can be visualized by light microscopy.

### Protocol Overview



## Virus Index

Virus Index	Product Name	Label	Product No.	Quantity	Page
HPV	ZytoFast HPV type 6/11 Probe	Biotin	T-1032-400	400 µl	225 f.
	ZytoFast HPV type 6/11 Probe C€ <input type="checkbox"/> IVD	Digoxigenin	T-1055-400	400 µl	225 f.
	ZytoFast HPV type 16/18 Probe	Biotin	T-1035-400	400 µl	225 f.
	ZytoFast HPV type 16/18 Probe C€ <input type="checkbox"/> IVD	Digoxigenin	T-1056-400	400 µl	225 f.
	ZytoFast HPV type 31/33 Probe	Biotin	T-1038-400	400 µl	225 f.
	ZytoFast HPV type 31/33 Probe C€ <input type="checkbox"/> IVD	Digoxigenin	T-1057-400	400 µl	225 f.
	ZytoFast HPV High-Risk (HR) Types Probe C€ <input type="checkbox"/> IVD (specific for HPV type 16/18/31/33/35/39/45/51/52/56/58/59/66/68/82)	Digoxigenin	T-1140-400	400 µl	225 f.
	ZytoFast HPV Screening Probe C€ <input type="checkbox"/> IVD (specific for HPV type 6/11/16/18/31/33/35/39/45/51/52/56/58/59/66/68/82)	Digoxigenin	T-1144-400	400 µl	225 f.
EBV (a.k.a. HHV-4)	ZytoFast EBV Probe	Biotin	T-1014-400	400 µl	227 f.
	ZytoFast EBV Probe C€ <input type="checkbox"/> IVD	Digoxigenin	T-1114-400	400 µl	227 f.
CMV (a.k.a. HHV-5)	ZytoFast CMV Probe	Biotin	T-1013-400	400 µl	229 f.
	ZytoFast CMV Probe	Digoxigenin	T-1113-400	400 µl	229 f.

## mRNA Index

mRNA Index	Product Name	Label	Product No.	Quantity	Page
Ig-kappa	ZytoFast human Ig-kappa Probe	Biotin	T-1015-400	400 µl	231 f.
	ZytoFast human Ig-kappa Probe C€ <input type="checkbox"/> IVD	Digoxigenin	T-1115-400	400 µl	231 f.
	ZytoFast human Ig-kappa/Ig-lambda Probe C€ <input type="checkbox"/> IVD	Digoxigenin/Biotin	T-1017-400	400 µl	231 f.
	ZytoFast human Ig-kappa/Ig-lambda CISH Kit C€ <input type="checkbox"/> IVD	Digoxigenin/Biotin	T-1005-40	40 Tests	231 f.
	ZytoFast human Ig-kappa/Ig-lambda Permanent CISH Kit C€ <input type="checkbox"/> IVD	Digoxigenin/Biotin	T-1105-40	40 Tests	231 f.
Ig-lambda	ZytoFast human Ig-lambda Probe	Biotin	T-1016-400	400 µl	231 f.
	ZytoFast human Ig-lambda Probe C€ <input type="checkbox"/> IVD	Digoxigenin	T-1116-400	400 µl	231 f.
	ZytoFast human Ig-kappa/Ig-lambda Probe C€ <input type="checkbox"/> IVD	Digoxigenin/Biotin	T-1017-400	400 µl	231 f.
	ZytoFast human Ig-kappa/Ig-lambda CISH Kit C€ <input type="checkbox"/> IVD	Digoxigenin/Biotin	T-1005-40	40 Tests	231 f.
	ZytoFast human Ig-kappa/Ig-lambda Permanent CISH Kit C€ <input type="checkbox"/> IVD	Digoxigenin/Biotin	T-1105-40	40 Tests	231 f.

C€  IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.

## Probes Sorted by Indication

Indication	Product Name	Label	Product No.	Quantity	Page
<b>Solid Tumors</b> Cervical Cancer	ZytoFast HPV type 6/11 Probe	Biotin	T-1032-400	400 µl	225 f.
	ZytoFast HPV type 6/11 Probe CE <span style="border: 1px solid black; padding: 0 2px;">IVD</span>	Digoxigenin	T-1055-400	400 µl	225 f.
	ZytoFast HPV type 16/18 Probe	Biotin	T-1035-400	400 µl	225 f.
	ZytoFast HPV type 16/18 Probe CE <span style="border: 1px solid black; padding: 0 2px;">IVD</span>	Digoxigenin	T-1056-400	400 µl	225 f.
	ZytoFast HPV type 31/33 Probe	Biotin	T-1038-400	400 µl	225 f.
	ZytoFast HPV type 31/33 Probe CE <span style="border: 1px solid black; padding: 0 2px;">IVD</span>	Digoxigenin	T-1057-400	400 µl	225 f.
	ZytoFast HPV High-Risk (HR) Types Probe CE <span style="border: 1px solid black; padding: 0 2px;">IVD</span> (specific for HPV type 16/18/31/33/35/39/45/51/52/56/58/59/66/68/82)	Digoxigenin	T-1140-400	400 µl	225 f.
	ZytoFast HPV Screening Probe CE <span style="border: 1px solid black; padding: 0 2px;">IVD</span> (specific for HPV type 6/11/16/18/31/33/35/39/45/51/52/56/58/59/66/68/82)	Digoxigenin	T-1144-400	400 µl	225 f.
	<b>Hematology</b> Lymphoma	ZytoFast EBV Probe	Biotin	T-1014-400	400 µl
ZytoFast EBV Probe CE <span style="border: 1px solid black; padding: 0 2px;">IVD</span>		Digoxigenin	T-1114-400	400 µl	227 f.
ZytoFast human Ig-kappa Probe		Biotin	T-1015-400	400 µl	231 f.
ZytoFast human Ig-kappa Probe CE <span style="border: 1px solid black; padding: 0 2px;">IVD</span>		Digoxigenin	T-1115-400	400 µl	231 f.
ZytoFast human Ig-lambda Probe		Biotin	T-1016-400	400 µl	231 f.
ZytoFast human Ig-lambda Probe CE <span style="border: 1px solid black; padding: 0 2px;">IVD</span>		Digoxigenin	T-1116-400	400 µl	231 f.
ZytoFast human Ig-kappa/Ig-lambda Probe CE <span style="border: 1px solid black; padding: 0 2px;">IVD</span>		Digoxigenin/Biotin	T-1017-400	400 µl	231 f.
ZytoFast human Ig-kappa/Ig-lambda CISH Kit CE <span style="border: 1px solid black; padding: 0 2px;">IVD</span>		Digoxigenin/Biotin	T-1005-40	40 Tests	231 f.
ZytoFast human Ig-kappa/Ig-lambda Permanent CISH Kit CE <span style="border: 1px solid black; padding: 0 2px;">IVD</span>		Digoxigenin/Biotin	T-1105-40	40 Tests	231 f.

CE IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.

## ZytoFast<sup>®</sup> HPV-CISH System

### Background

The ZytoFast<sup>®</sup> HPV-CISH System is designed for the detection and discrimination of human papilloma virus (HPV) DNA in paraffin-embedded tissue sections or cell samples.

At least 50 percent of sexually active men and women acquire some form of genital HPV infection at some point in their lives. Most of the approx. 30 identified genital HPV types, predominantly types 6 and 11, are called "low-risk" types, and may cause mild Pap test abnormalities or genital warts.

Until now, approximately 10–15 HPV types are associated with lesions that can progress to cancer. Among those are the HPV types 16/18/31/33/35/39/45/51/52/56/58/59/66/68/82.

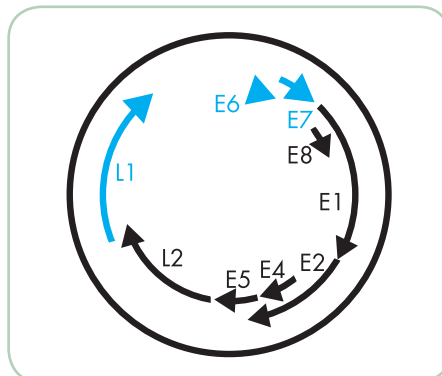
These cancer-associated HPV types are designated as high-risk HPV (hr-HPV) types. The infection with the HPV hr-types can lead to development of cancer of the cervix, vulva, vagina, anus, or penis. The majority of malignant cervical carcinomas (approx. 70%) occur as a result of infections with HPV types 16 or 18.

### References

- Cothran MM & White JP [2002] Health Care Women Int 23: 306-19.  
 Cubie HA & Norval M (1988) J Virol Methods 20: 239-49.  
 Faulkner-Jones BE, et al. (1993) J Virol Methods 41: 277-96.  
 Francis IM, et al. (2013) Sultan Qaboos Univ Med J 13: 527-33.  
 Grundmeier N, et al. (2011) Dermatology 223: 293-300.  
 Kaspersen MD, et al. (2011) PLoS One 6: e18095.  
 Mirasoli M, et al. (2009) Anal Bioanal Chem 394: 981-7.  
 Montag M, et al. (2011) Arch Gynecol Obstet 284: 999-1005.  
 Poljak M & Kocjan BJ (2010) Expert Rev Anti Infect Ther 8: 1139-62.  
 Reinholz M, et al. (2013) Arch Dermatol Res 305: 723-32.  
 Walboomers JMM, et al. (1999) J Pathol 189: 12-9.

### Probe Description

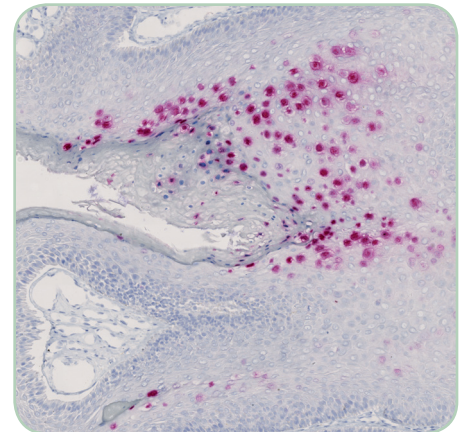
ZytoFast<sup>®</sup> HPV specific probes are directed against DNA sequences which encode the HPV proteins E6, E7 and/or L1. The probes consist of HPV-type-specific oligonucleotides, Biotin or Digoxigenin labeled by using the unique ZytoFast<sup>®</sup> HighTag System providing improved signal intensity. In addition to the detection of HPV at the DNA level, HPV probes will also allow detection of E6, E7, and/or L1 RNAs, which are expressed during some stages of infection.



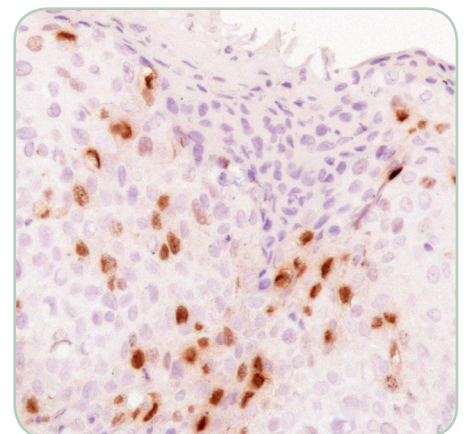
Schematic representation of the HPV genome with E and L open reading frames. Genomic regions targeted by ZytoFast<sup>®</sup> HPV specific oligonucleotides are indicated in blue.

### Results

A positive reactivity for HPV DNA in epithelial cells is indicated by a distinctly stained nucleus. Due to the detection of HPV DNA as well as E6, E7, and/or L1 RNAs, depending on the infection stage, cytoplasmic staining might be observed additionally. Depending on the detection chemistry that is used, colored precipitates, which can be clearly distinguished from the background, will be dark violet-blue when using NBT/BCIP as substrate, dark brown when using DAB, or strong red when using Permanent Red.



HPV infected cervix tissue hybridized with the ZytoFast<sup>®</sup> HPV type 6/11 Probe, detected with the ZytoFast<sup>®</sup> PLUS CISH Implementation Kit AP-Permanent Red.



HPV infected cervix tissue hybridized with the ZytoFast<sup>®</sup> HPV High-Risk (HR) Types Probe, detected with the ZytoFast<sup>®</sup> PLUS CISH Implementation Kit HRP-DAB.

## ZytoFast<sup>®</sup> HPV Probes

Biotin-labeled

Prod. No.	Product	Tests* (Volume)
T-1032-400	ZytoFast HPV type 6/11 Probe	40 (400 µl)
T-1035-400	ZytoFast HPV type 16/18 Probe	40 (400 µl)
T-1038-400	ZytoFast HPV type 31/33 Probe	40 (400 µl)
<b>Related Products</b>		
T-1070-40	ZytoFast CISH Implementation Kit AP-NBT/BCIP Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 2x 50 ml; AP-Streptavidin, 4 ml; NBT/BCIP Solution, 4 ml	40

## ZytoFast<sup>®</sup> HPV-Probes

Digoxigenin-labeled

Prod. No.	Product	Tests* (Volume)
T-1055-400	ZytoFast HPV type 6/11 Probe CE <input type="checkbox"/> IVD	40 (400 µl)
T-1056-400	ZytoFast HPV type 16/18 Probe CE <input type="checkbox"/> IVD	40 (400 µl)
T-1057-400	ZytoFast HPV type 31/33 Probe CE <input type="checkbox"/> IVD	40 (400 µl)
T-1140-400	ZytoFast HPV High-Risk (HR) Types Probe (specific for HPV type 16/18/31/33/35/39/45/51/52/56/58/59/66/68/82) CE <input type="checkbox"/> IVD	40 (400 µl)
T-1144-400	ZytoFast HPV Screening Probe (specific for HPV type 6/11/16/18/31/33/35/39/45/51/52/56/58/59/66/68/82) CE <input type="checkbox"/> IVD	40 (400 µl)
<b>Related Products</b>		
T-1061-40	ZytoFast PLUS CISH Implementation Kit AP-NBT/BCIP CE <input type="checkbox"/> IVD Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x50 ml; Rabbit-anti-DIG, 4 ml; Anti-Rabbit-AP-Polymer, 4 ml; NBT/BCIP Solution, 4ml; Nuclear Red Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40
T-1063-40	ZytoFast PLUS CISH Implementation Kit HRP-DAB CE <input type="checkbox"/> IVD Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Mouse-anti-DIG, 4 ml; Anti-Mouse-HRP-Polymer, 4 ml; DAB Solution A, 0.3 ml; DAB Solution B, 10 ml; Nuclear Blue Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40
T-1151-40	ZytoFast PLUS CISH Implementation Kit AP-Permanent Red CE <input type="checkbox"/> IVD Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x50 ml; Rabbit-anti-DIG, 4 ml; Anti-Rabbit-AP-Polymer, 4 ml; Permanent Red Solution A, 0.25 ml; Permanent Red Solution B, 15 ml; Mayer's Hematoxylin Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40

\* Using 10 µl probe solution per test. CE  IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.

## ZytoFast<sup>®</sup> EBV-CISH System

### Background

The ZytoFast<sup>®</sup> EBV-CISH System is designed for the detection of Epstein-Barr virus (EBV) RNA in paraffin-embedded tissue sections or cell samples.

EBV (a.k.a. human herpesvirus-4, HHV-4) is a member of the gamma-herpesvirus group and one of the most common viruses in humans.

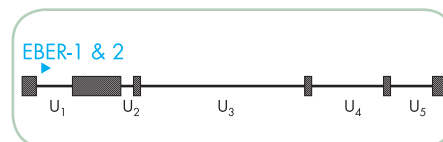
Transmission of EBV requires close, intimate contact with a person excreting the virus in its saliva. EBV has two major target tissues *in vivo*, B lymphocytes and squamous pharyngeal epithelium. Infection of B lymphocytes with EBV results in persistent latent infection, immortalization of the cells, and perpetual proliferation. EBV, the first virus to be identified as an oncovirus, is the etiological agent of infectious mononucleosis and has been implicated in the pathogenesis of an increasing number of human malignancies such as Burkitt's lymphoma, nasopharyngeal carcinoma, and polyclonal lymphomas in immunocompromised individuals. CISH-based diagnosis of EBV infection has the advantage over other methods in that it permits unequivocal localization of EBV genomes in cells and thereby obviates the risk of false positive results due to laboratory or clinical contamination.

### References

- Agaimy A & Wünsch PH (2006) *Pathol Res Pract* 202: 541-8.  
 Fuchs S, et al. (2012) *J Immunol* 88: 1523-33.  
 Gaiser T, et al. (2012) *Diagn Pathol* 7: 38.  
 Greifenegger N, et al. (1998) *J Virol* 72: 9323-8.  
 Khan G, et al. (1992) *J Clin Pathol* 45: 616-20.  
 Kim DN, et al. (2013) *J Gen Virol* 94: 497-506.  
 Murphy JK, et al. (1990) *J Clin Pathol* 43: 220-3.  
 Rosa MD, et al. (1981) *Mol Cell Biol* 1: 785-96.  
 Sides MD, et al. (2013) *Virol J* 10: 152.  
 Thorley-Lawson DA (2001) *Nat Rev Immunol* 1: 75-82.

### Probe Description

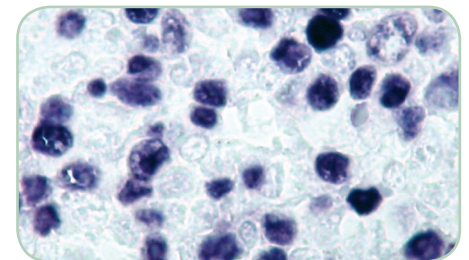
The ZytoFast<sup>®</sup> EBV Probe is directed against EBER-1 and EBER-2 RNA sequences that were found to be transcribed in every latently infected cell. Due to the large number (up to 10<sup>7</sup>) of copies per cell, these RNAs are the most abundant transcripts in latently EBV-infected cells. The probe consists of EBV-specific oligonucleotides, Biotin or Digoxigenin labeled by using the unique ZytoFast<sup>®</sup> High Tag System providing improved signal intensity.



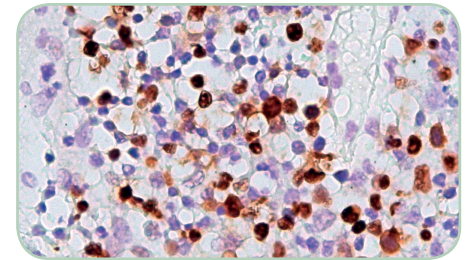
Schematic representation of the EBV genome with the EBER-1 and EBER-2 encoding region indicated in blue. U1-U5 indicate unique nucleotide sequences, hatched boxes represent terminal and internal repeats.

### Results

A positive reactivity for Epstein-Barr-Virus (EBV) EBER RNA in the target cells is indicated by a distinctly stained nucleus. Depending on the detection chemistry that is used, colored precipitates, which can be clearly distinguished from the background, will be dark violet-blue when using NBT/BCIP as substrate, dark brown when using DAB, or strong red when using Permanent Red.



CISH analysis of paraffin-embedded tonsil tissue using the ZytoFast<sup>®</sup> EBV Probe, detected with ZytoFast<sup>®</sup> CISH Implementation Kit AP-NBT/BCIP.



EBV infected tonsil tissue hybridized with ZytoFast<sup>®</sup> EBV Probe, detected with ZytoFast<sup>®</sup> PLUS CISH Implementation Kit HRP-DAB.

## ZytoFast<sup>®</sup> EBV-CISH Probes

Biotin-labeled

Prod. No.	Product	Tests* (Volume)
T-1014-400	ZytoFast EBV Probe	40 (400 µl)
<b>Related Products</b>		
T-1070-40	ZytoFast CISH Implementation Kit AP-NBT/BCIP Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 2x 50 ml; AP-Streptavidin, 4 ml; NBT/BCIP Solution, 4 ml	40

## ZytoFast<sup>®</sup> EBV-CISH Probes

Digoxigenin-labeled

Prod. No.	Product	Tests* (Volume)
T-1114-400	ZytoFast EBV Probe C€ <span style="border: 1px solid black; padding: 0 2px;">IVD</span>	40 (400 µl)
<b>Related Products</b>		
T-1061-40	ZytoFast PLUS CISH Implementation Kit AP-NBT/BCIP C€ <span style="border: 1px solid black; padding: 0 2px;">IVD</span> Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Rabbit-anti-DIG, 4 ml; Anti-Rabbit-AP-Polymer, 4 ml; NBT/BCIP Solution, 4ml; Nuclear Red Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40
T-1063-40	ZytoFast PLUS CISH Implementation Kit HRP-DAB C€ <span style="border: 1px solid black; padding: 0 2px;">IVD</span> Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Mouse-anti-DIG, 4 ml; Anti-Mouse-HRP-Polymer, 4 ml; DAB Solution A, 0.3 ml; DAB Solution B, 10 ml; Nuclear Blue Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40
T-1151-40	ZytoFast PLUS CISH Implementation Kit AP-Permanent Red C€ <span style="border: 1px solid black; padding: 0 2px;">IVD</span> Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Rabbit-anti-DIG, 4 ml; Anti-Rabbit-AP-Polymer, 4 ml; Permanent Red Solution A, 0.25 ml; Permanent Red Solution B, 15 ml; Mayer's Hematoxylin Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40

\* Using 10 µl probe solution per test. C€ IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.



## ZytoFast<sup>®</sup> CMV-CISH System

### Background

The ZytoFast<sup>®</sup> CMV-CISH System is designed for the detection of cytomegalovirus (CMV) DNA in paraffin-embedded tissue sections or cell samples. CMV (a.k.a. human herpesvirus-5, HHV-5) is a member of the beta-herpesvirus group and may be found in 40-100% of people. CMV can be transmitted sexually as well as via breast milk, transplanted organs, and rarely from blood transfusions.

Following primary CMV infection in the normal host, the virus remains in a latent state and can be found in multiple body sites as it is, unlike other herpesviruses, not restricted to certain body areas. Among immunosuppressed patients, such as recipients of solid organ or haematopoietic stem cell allografts, CMV infections are common causes of morbidity and mortality.

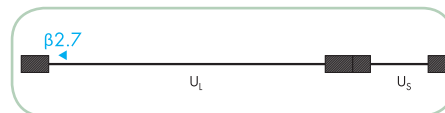
In histology, the hallmark of CMV infection is the finding of intranuclear inclusions consistent with the virus. CISH-based diagnosis of CMV infection has the advantage over other methods in that it permits unequivocal localization of CMV genomes in cells and thereby obviates the risk of false positive results due to laboratory or clinical contamination.

### References

Cobbs CS (2002) *Cancer Res* 62: 3347-50.  
Chen HP, et al. (2012) *J Clin Virol* 54: 240-4.  
Greenaway PJ & Wilkinson GWG (1987) *Virus Res* 7: 17-31.  
Spector SA (1990) *Semin Hematol* 27 (2 Suppl 1): 11-6; 28-9.  
Wu TC, et al. (1992) *Am J Pathol* 141: 1247-54.

### Probe Description

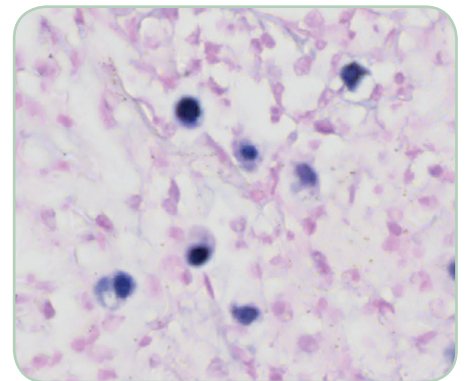
The ZytoFast<sup>®</sup> CMV Probe is directed against the sequence of the  $\beta 2.7$  gene, the most abundantly transcribed early CMV gene. The probe consists of CMV-specific oligonucleotides, Biotin or Digoxigenin labeled by using the unique ZytoFast<sup>®</sup> HighTag System providing improved signal intensity. In addition to the detection of CMV at the DNA level, the CMV Probe will also allow detection of the  $\beta 2.7$  RNA, which is expressed during all stages of infection.



Schematic representation of the CMV genome with the  $\beta 2.7$  encoding region indicated in blue. UL and US indicate unique nucleotide sequences, hatched boxes represent terminal and internal repeats.

### Results

Due to the detection of CMV DNA as well as of the abundantly transcribed  $\beta 2.7$  RNA, a positive reactivity for cytomegalovirus (CMV) in the target cells is indicated by a cytoplasmic and/or nuclear staining pattern. Depending on the detection chemistry that is used, colored precipitates, which can be clearly distinguished from the background, will be dark violet-blue when using NBT/BCIP as substrate, dark brown when using DAB, or strong red when using Permanent Red.



CISH analysis of paraffin-embedded adrenal gland tissue using the ZytoFast<sup>®</sup> CMV Probe, detected with ZytoFast<sup>®</sup> PLUS CISH Implementation Kit AP-NBT/BCIP.

## ZytoFast<sup>®</sup> CMV-CISH Probes

Biotin-labeled

Prod. No.	Product	Tests* (Volume)
T-1013-400	ZytoFast CMV Probe	40 (400 µl)
<b>Related Products</b>		
T-1070-40	ZytoFast CISH Implementation Kit AP-NBT/BCIP Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 2x 50 ml; AP-Streptavidin, 4 ml; NBT/BCIP Solution, 4 ml	40

## ZytoFast<sup>®</sup> CMV-CISH Probes

Digoxigenin-labeled

Prod. No.	Product	Tests* (Volume)
T-1113-400	ZytoFast CMV Probe	40 (400 µl)
<b>Related Products</b>		
T-1061-40	ZytoFast PLUS CISH Implementation Kit AP-NBT/BCIP <b>CE</b> <b>IVD</b> Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Rabbit-anti-DIG, 4 ml; Anti-Rabbit-AP-Polymer, 4 ml; NBT/BCIP Solution, 4 ml; Nuclear Red Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40
T-1063-40	ZytoFast PLUS CISH Implementation Kit HRP-DAB <b>CE</b> <b>IVD</b> Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Mouse-anti-DIG, 4 ml; Anti-Mouse-HRP-Polymer, 4 ml; DAB Solution A, 0.3 ml; DAB Solution B, 10 ml; Nuclear Blue Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40
T-1151-40	ZytoFast PLUS CISH Implementation Kit AP-Permanent Red <b>CE</b> <b>IVD</b> Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Rabbit-anti-DIG, 4 ml; Anti-Rabbit-AP-Polymer, 4 ml; Permanent Red Solution A, 0.25 ml; Permanent Red Solution B, 15 ml; Mayer's Hematoxylin Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40

\* Using 10 µl probe solution per test. **CE** **IVD** only available in certain countries. All other countries research use only! Please contact your local dealer for more information.

## ZytoFast<sup>®</sup> Ig-kappa/Ig-lambda-CISH System

### Background

The ZytoFast<sup>®</sup> Ig-kappa/Ig-lambda-CISH System is designed for the detection of Ig-kappa ( $\kappa$ ) and/or Ig-lambda ( $\lambda$ ) light chain mRNA in paraffin-embedded tissue sections or cell samples.

B-cells (a.k.a. B lymphocytes) develop from lymphoid stem cells in the bone marrow. Each clone of B-cells expresses a unique antibody molecule, composed of 2 identical heavy and 2 identical light chains, the latter either of  $\kappa$  or  $\lambda$  type. Determination of kappa-to-lambda ratio is useful to distinguish between neoplastic and reactive lymphoid proliferations. Polyclonal expression of  $\kappa$  or  $\lambda$  light chains is considered to reflect a reactive hyperplasia in contrast to the monoclonal expression in malignant lymphoma, the most common hematologic malignancy encountered in the Western world. Whereas detection of Ig- $\kappa$  and Ig- $\lambda$  by immunohistochemistry often results in excessive background staining, *in situ* Hybridization has the advantage of a virtually background-free signal, allowing a safe and simple analysis of the clonality of a given lymphocyte population.

### References

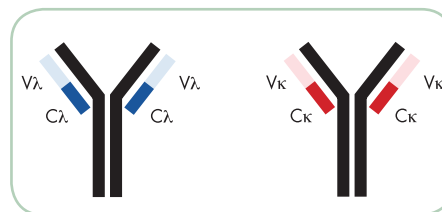
- Erber WN, et al. (1993) Pathology 25: 63-7.  
Ke L, et al. (2011) Int J Clin Exp Pathol 4: 190-6.  
McElroy MK, et al. (2011) Hum Pathol 42: 1813-8.  
McNicol AM & Farquharson MA (1997) J Pathol 182: 250-61.  
Pringle JH, et al. (1990) J Pathol 162: 197-207.

### Probe Description

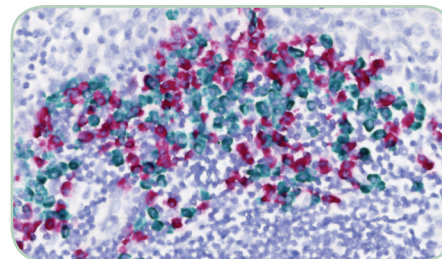
ZytoFast<sup>®</sup> human Ig-kappa Probe is directed against mRNA sequences encoding  $\kappa$  light chain constant regions of human immunoglobulins.

The ZytoFast<sup>®</sup> human Ig-lambda Probe is directed against mRNA sequences encoding  $\lambda$  light chain constant regions of human immunoglobulins.

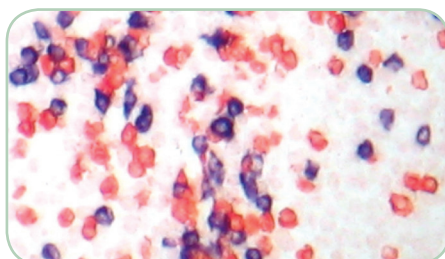
The ZytoFast<sup>®</sup> human Ig-kappa/Ig-lambda Probe is a probe mixture consisting of a Digoxigenin labeled Ig- $\kappa$  mRNA specific probe and a Biotin labeled Ig- $\lambda$  mRNA specific probe. All probes are tagged by using the unique ZytoFast<sup>®</sup> HighTag System providing improved signal intensity.



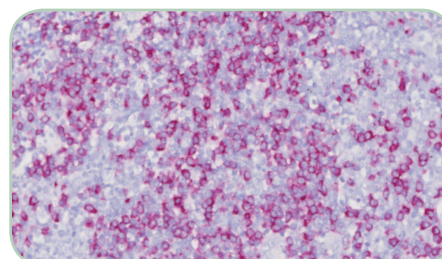
Basic immunoglobulin structure indicating the heavy chains (black),  $\lambda$  (blue) and  $\kappa$  (red) light chains. The light chain constant regions (C) whose encoding mRNA sequences are targeted by ZytoFast<sup>®</sup> Ig-lambda and Ig-kappa probes are indicated in dark blue and red respectively, the variable regions (V) in light blue and red.



CISH analysis of a paraffin-embedded tonsil tissue using the ZytoFast<sup>®</sup> human Ig-kappa/Ig-lambda Permanent CISH Kit.



CISH analysis of a paraffin-embedded bone marrow biopsy specimen using the ZytoFast<sup>®</sup> human Ig-kappa/Ig-lambda CISH Kit.



Tonsil tissue with B-cells expressing Ig-kappa hybridized with ZytoFast<sup>®</sup> human Ig-kappa Probe, detected with ZytoFast<sup>®</sup> PLUS CISH Implementation Kit AP-Permanent Red.

### Results

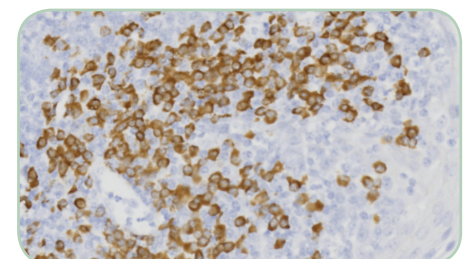
A positive reactivity in the target cells is indicated by cytoplasmic staining. Depending on the detection chemistry that is used, colored precipitates, which can be clearly distinguished from the background, will be dark violet-blue when using NBT/BCIP as substrate, strong red when using AEC, dark brown when using DAB, green when using HRP-Green, or strong red when using Permanent Red.

Using the ZytoFast<sup>®</sup> human Ig-kappa Probe, B-cells expressing antibodies with  $\kappa$  light chains will result in cytoplasmic staining whereas Ig- $\lambda$  expressing B-cells are not stained.

Using the ZytoFast<sup>®</sup> human Ig-lambda Probe, B-cells expressing antibodies with  $\lambda$  light chains will result in cytoplasmic staining whereas Ig- $\kappa$  expressing B-cells are not stained.

Using the ZytoFast<sup>®</sup> human Ig-kappa/Ig-lambda CISH Kit, B-cells expressing antibodies with  $\kappa$  light chains will result in a red cytoplasmic staining and simultaneously Ig- $\lambda$  expressing B-cells will result in a dark violet-blue cytoplasmic staining.

Using the ZytoFast<sup>®</sup> human Ig-kappa/Ig-lambda Permanent CISH Kit, B-cells expressing antibodies with  $\kappa$  light chains will result in a green cytoplasmic staining and simultaneously Ig- $\lambda$  expressing B-cells will result in permanent red cytoplasmic staining.



Tonsil tissue with B-cells expressing Ig-kappa hybridized with ZytoFast<sup>®</sup> human Ig-kappa Probe, detected with ZytoFast<sup>®</sup> PLUS CISH Implementation Kit HRP-DAB.

## ZytoFast<sup>®</sup> Ig-kappa/Ig-lambda Probes

Biotin-labeled

Prod. No.	Product	Tests* (Volume)
T-1015-400	ZytoFast human Ig-kappa Probe	40 (400 µl)
T-1016-400	ZytoFast human Ig-lambda Probe	40 (400 µl)
<b>Related Products</b>		
T-1070-40	ZytoFast CISH Implementation Kit AP-NBT/BCIP Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 2x 50 ml; AP-Streptavidin, 4 ml; NBT/BCIP Solution, 4 ml	40

## ZytoFast<sup>®</sup> Ig-kappa/Ig-lambda Probes

Digoxigenin-labeled

Prod. No.	Product	Tests* (Volume)
T-1115-400	ZytoFast human Ig-kappa Probe CE <input type="checkbox"/> IVD	40 (400 µl)
T-1116-400	ZytoFast human Ig-lambda Probe CE <input type="checkbox"/> IVD	40 (400 µl)
<b>Related Products</b>		
T-1061-40	ZytoFast PLUS CISH Implementation Kit AP-NBT/BCIP CE <input type="checkbox"/> IVD Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Rabbit-anti-DIG, 4 ml; Anti-Rabbit-AP-Polymer, 4 ml; NBT/BCIP Solution, 4 ml; Nuclear Red Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40
T-1063-40	ZytoFast PLUS CISH Implementation Kit HRP-DAB CE <input type="checkbox"/> IVD Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Mouse-anti-DIG, 4 ml; Anti-Mouse-HRP-Polymer, 4 ml; DAB Solution A, 0.3 ml; DAB Solution B, 10 ml; Nuclear Blue Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40
T-1151-40	ZytoFast PLUS CISH Implementation Kit AP-Permanent Red CE <input type="checkbox"/> IVD Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Rabbit-anti-DIG, 4 ml; Anti-Rabbit-AP-Polymer, 4 ml; Permanent Red Solution A, 0.25 ml; Permanent Red Solution B, 15 ml; Mayer's Hematoxylin Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40

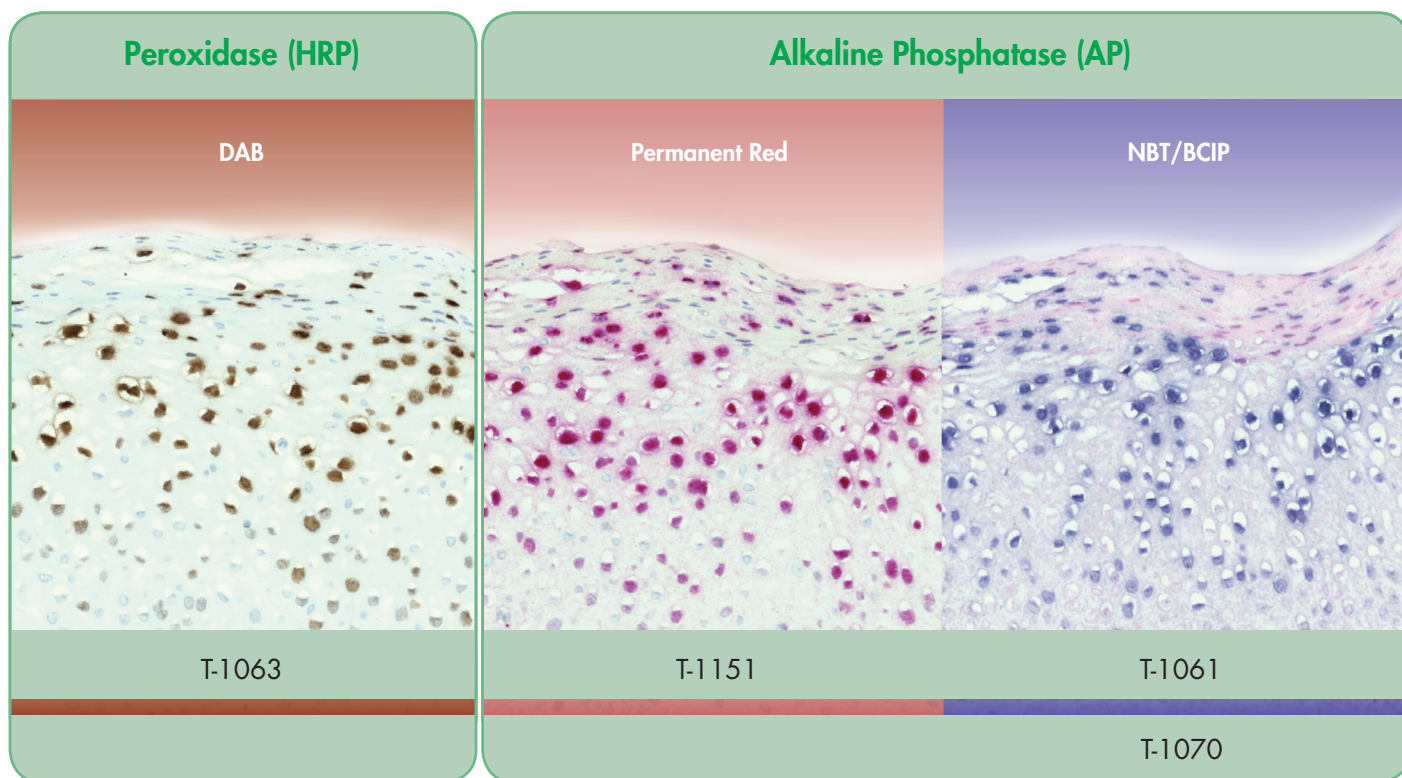
## ZytoFast<sup>®</sup> Ig-kappa/Ig-lambda Probes

Biotin/Digoxigenin-labeled

Prod. No.	Product	Tests* (Volume)
T-1017-400	ZytoFast human Ig-kappa/Ig-lambda Probe CE <input type="checkbox"/> IVD	40 (400 µl)
<b>Related Products</b>		
T-1005-40	ZytoFast human Ig-kappa/Ig-lambda CISH Kit CE <input type="checkbox"/> IVD Incl. Ig-kappa/Ig-lambda Probe (Digoxigenin/Biotin labeled), 0.4 ml; 28S rRNA (+) Control Probe (Biotin labeled), 0.1 ml; RNA (-) Control Probe (Biotin labeled), 0.1 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 2x 50 ml; HRP-anti-Digoxigenin, 4 ml; AP-Streptavidin, 4 ml; AEC Solution, 4 ml; NBT/BCIP Solution, 4 ml	40
T-1105-40	ZytoFast human Ig-kappa/Ig-lambda Permanent CISH Kit CE <input type="checkbox"/> IVD Incl. Ig-kappa/Ig-lambda Probe (Digoxigenin/Biotin labeled), 0.4 ml; 28S rRNA (+) Control Probe (Biotin labeled), 0.1 ml; RNA (-) Control Probe (Biotin labeled), 0.1 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 2x 50 ml; HRP-anti-Digoxigenin, 4 ml; AP-Streptavidin, 4 ml; HRP-Green-Solution A, 0.8 ml; HRP-Green-Solution B, 15 ml; Permanent Red Solution A, 0.25 ml; Permanent Red Solution B, 15 ml; Nuclear Blue Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	40

\* Using 10 µl probe solution per test. CE  IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.

## Accessories



## ZytoFast<sup>®</sup> PLUS Implementation Kits

For the detection of Digoxigenin-labeled ZytoFast<sup>®</sup> Probes

Prod. No.	Product	Tests
T-1061-40	ZytoFast PLUS CISH Implementation Kit AP-NBT/BCIP CE <input type="checkbox"/> IVD	40
Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Rabbit-anti-DIG, 4 ml; Anti-Rabbit-AP-Polymer, 4 ml; NBT/BCIP Solution, 4ml; Nuclear Red Solution, 20 ml; Mounting Solution (alcoholic), 4 ml		
T-1063-40	ZytoFast PLUS CISH Implementation Kit HRP-DAB CE <input type="checkbox"/> IVD	40
Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Mouse-anti-DIG, 4 ml; Anti-Mouse-HRP-Polymer, 4 ml; DAB Solution A, 0.3 ml; DAB Solution B, 10 ml; Nuclear Blue Solution, 20 ml; Mounting Solution (alcoholic), 4 ml		
T-1151-40	ZytoFast PLUS CISH Implementation Kit AP-Permanent Red CE <input type="checkbox"/> IVD	40
Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 4x 50 ml; Rabbit-anti-DIG, 4 ml; Anti-Rabbit-AP-Polymer, 4 ml; Permanent Red Solution A, 0.25 ml; Permanent Red Solution B, 1.5 ml; Mayer's Hematoxylin Solution, 20 ml; Mounting Solution (alcoholic), 4 ml		

## ZytoFast<sup>®</sup> Implementation Kits

For the detection of Biotin-labeled ZytoFast<sup>®</sup> Probes

Prod. No.	Product	Tests
T-1070-40	ZytoFast CISH Implementation Kit AP-NBT/BCIP	40
Incl. DNA (+) Control Probe, 0.1 ml; DNA (-) Control Probe, 0.1 ml; 28S rRNA (+) Control Probe, 0.1 ml; RNA (-) Control Probe, 0.1 ml; Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 2x 50 ml; AP-Streptavidin, 4 ml; NBT/BCIP, 4 ml		

CE  IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.

## Accessories

### ZytoFast<sup>®</sup> Pretreatment Reagents

Prod. No.	Product
ES-0001-4	Pepsin Solution, 4 ml C€ <span>IVD</span>
ES-0001-8	Pepsin Solution Set, 2x 4 ml C€ <span>IVD</span>
ES-0001-50	Pepsin Solution, 50 ml C€ <span>IVD</span>
ES-0001-1000	Pepsin Solution, 1000 ml C€ <span>IVD</span>
PT-0002-500	Heat Pretreatment Solution EDTA, 500 ml C€ <span>IVD</span>

### ZytoFast<sup>®</sup> Wash Buffers & Ancillary Reagents

Prod. No.	Product
AB-0001-4	Mouse-anti-DIG, 4 ml C€ <span>IVD</span>
AB-0001-30	Mouse-anti-DIG, 30 ml C€ <span>IVD</span>
AB-0002-4	Anti-Mouse-HRP-Polymer, 4 ml C€ <span>IVD</span>
AB-0008-4	HRP-anti-Digoxigenin, 4 ml C€ <span>IVD</span>
AB-0009-4	AP-Streptavidin, 4 ml C€ <span>IVD</span>
AB-0011-4	Rabbit-anti-DIG, 4 ml C€ <span>IVD</span>
AB-0012-4	Anti-Rabbit-AP-Polymer, 4 ml C€ <span>IVD</span>
C-3015-100	DAB Solution Set C€ <span>IVD</span> Incl. DAB Solution A, 0.3 ml; DAB Solution B, 10 ml; good for 10 ml DAB Solution
C-3039-100	ZytoDot HRP-Green Solution Set C€ <span>IVD</span> Incl. HRP-Green Solution A, 0.8 ml; HRP-Green Solution B, 15 ml; good for 15 ml HRP-Green Solution
CS-0001-20	Mayer's Hematoxylin Solution, 20 ml C€ <span>IVD</span>
CS-0002-20	Nuclear Blue Solution, 20 ml C€ <span>IVD</span>
CS-0003-20	Nuclear Red Solution, 20 ml C€ <span>IVD</span>
E-4005-50	Fixogum, Rubber Cement, 50 g
E-4005-125	Fixogum, Rubber Cement, 125 g
SB-0004-4	NBT/BCIP Solution, 4 ml C€ <span>IVD</span>
SB-0005-4	AEC Solution, 4 ml C€ <span>IVD</span>
T-1006-40	ZytoFast AP-Streptavidin Detection Kit, Incl. NBT/BCIP, 4 ml; AP-Streptavidin, 4ml
WB-0005-50	20x Wash Buffer TBS, 50 ml C€ <span>IVD</span>
WB-0006-0.5	2x Oligo Buffer RNA, 0.5 ml; good for 1 ml customer made labeled oligonucleotides

### ZytoFast<sup>®</sup> Control Probes

Biotin-labeled

Prod. No.	Product	Tests* (Volume)
T-1022-100	ZytoFast DNA (+) Control Probe	10 (100 µl)
T-1023-100	ZytoFast DNA (-) Control Probe	10 (100 µl)
T-1020-100	ZytoFast 28S rRNA (+) Control Probe C€ <span>IVD</span>	10 (100 µl)
T-1019-100	ZytoFast RNA (-) Control Probe C€ <span>IVD</span>	10 (100 µl)

### ZytoFast<sup>®</sup> Control Probes

Digoxigenin-labeled

Prod. No.	Product	Tests* (Volume)
T-1053-400	ZytoFast DNA (+) Control Probe C€ <span>IVD</span>	40 (400 µl)
T-1054-400	ZytoFast DNA (-) Control Probe C€ <span>IVD</span>	40 (400 µl)
T-1120-400	ZytoFast 28S rRNA (+) Control Probe C€ <span>IVD</span>	40 (400 µl)
T-1119-400	ZytoFast RNA (-) Control Probe C€ <span>IVD</span>	40 (400 µl)

\* Using 10 µl probe solution per test. C€ IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.