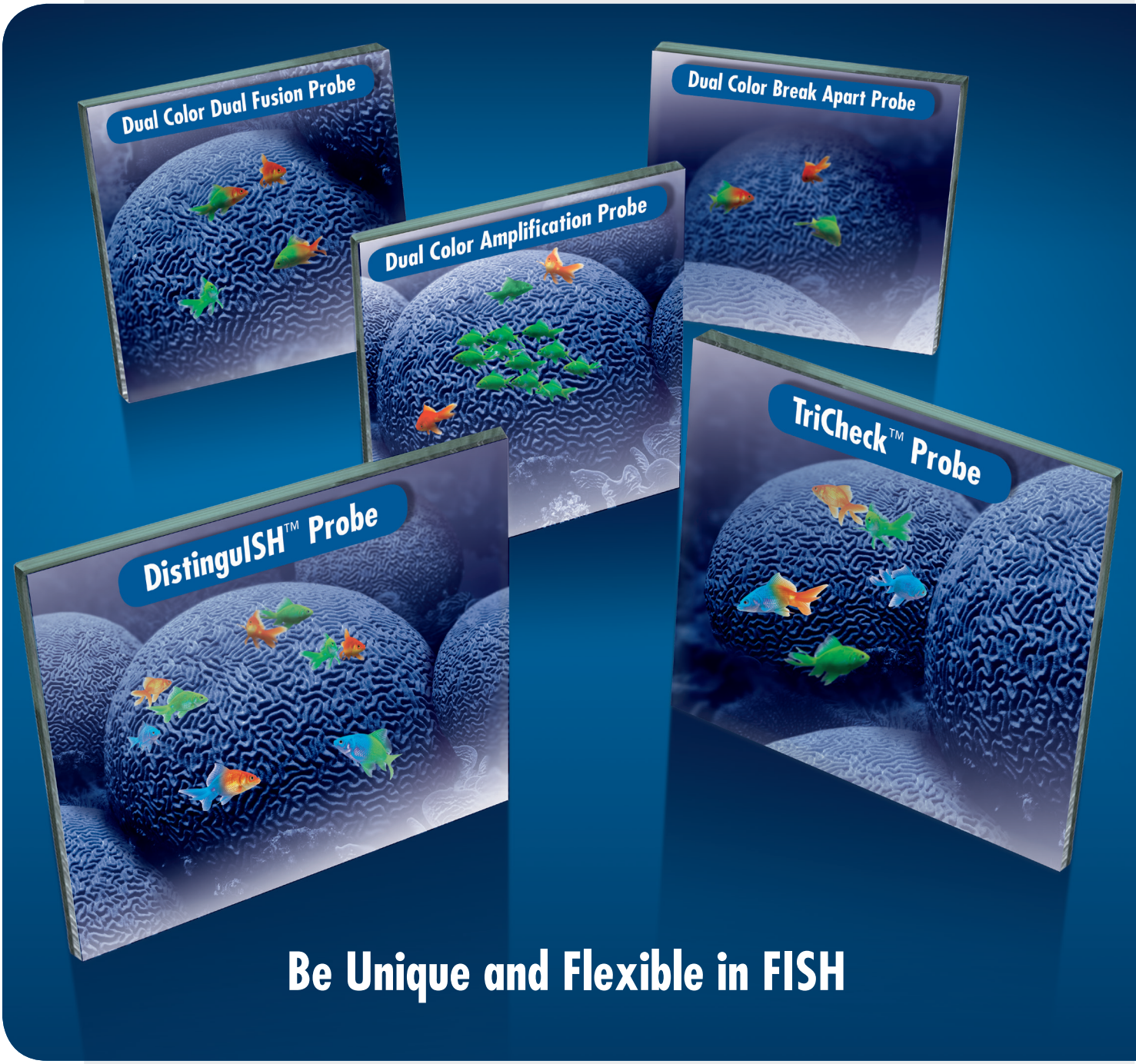
CE IVD

ZytoLight® Products for FISH analysis

FlexISH® Products for flexible FISH

ZytoMation® Products for automated FISH

FISH Signal Evaluation Guideline



Be Unique and Flexible in FISH

ZYTOVISION
Molecular diagnostics simplified

FISH Probes

ZytoLight® FlexISH® ZytoMation®

ZytoLight®, **FlexISH®**, and **ZytoMation®** products are designed for identification of chromosomal aberrations (e.g. translocations, deletions, amplifications, and chromosomal aneuploidies) on various specimens by FISH. **ZytoVision's FISH probes** are direct labeled and ready-to-use. **ZytoLight®** with its extensive portfolio provides a variety of unique and innovative probe designs for differentiated genetic analysis. **FlexISH®** products give the customer the flexibility to choose between a 1-day (2 h hybridization) or a 2-day (overnight hybridization) protocol by adapting the hybridization time to the customer's needs. **ZytoMation®** provides FISH probes for automated application on selected stainer systems.

Overview Probe Designs

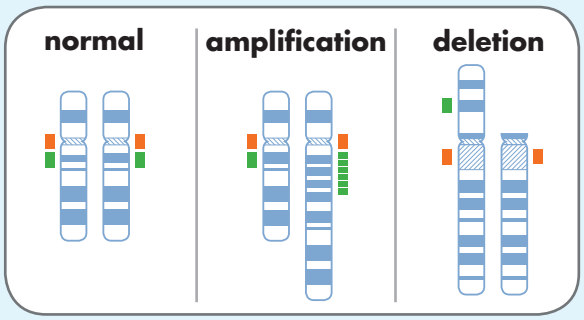
	ZytoLight®	FlexISH®	ZytoMation®
Dual Color Amplification/Deletion	x	x	x
Dual Color Dual Fusion	x		
Dual Color Break Apart	x		x
TriCheck™	x	x	
DistinguISH™		x	

Dual Color Probe Design

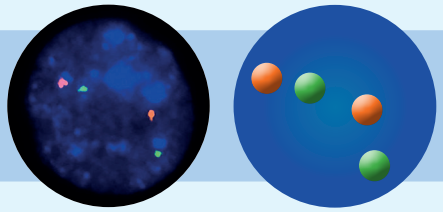
ZytoLight® FlexISH® ZytoMation®

e.g. ZytoLight® SPEC ERBB2/CEN 17 Dual Color Probe

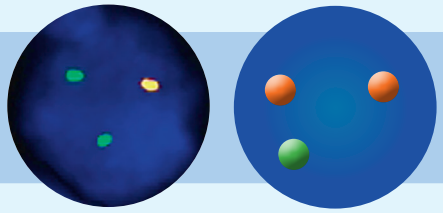
Dual Color Probes consist of a mixture of a green fluorochrome direct labeled SPEC probe hybridizing to the gene of interest and an orange fluorochrome direct labeled CEN or SPEC probe hybridizing to the centromeric region or a chromosome specific locus.
This two-color detection is especially useful for the differentiation of aneusomy from gene amplification and gene deletion.



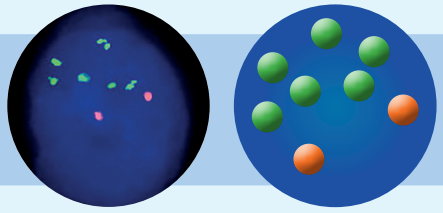
Signal Pattern



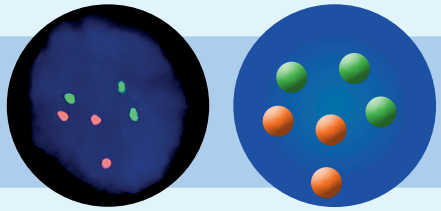
Normal — **Two single green and two single orange signals.**



Deletion — **One single green signal and two orange signals.**



Amplification — **Multiple green signals and two single orange signals.**



Aneusomy — **Three single green and three single orange signals.**

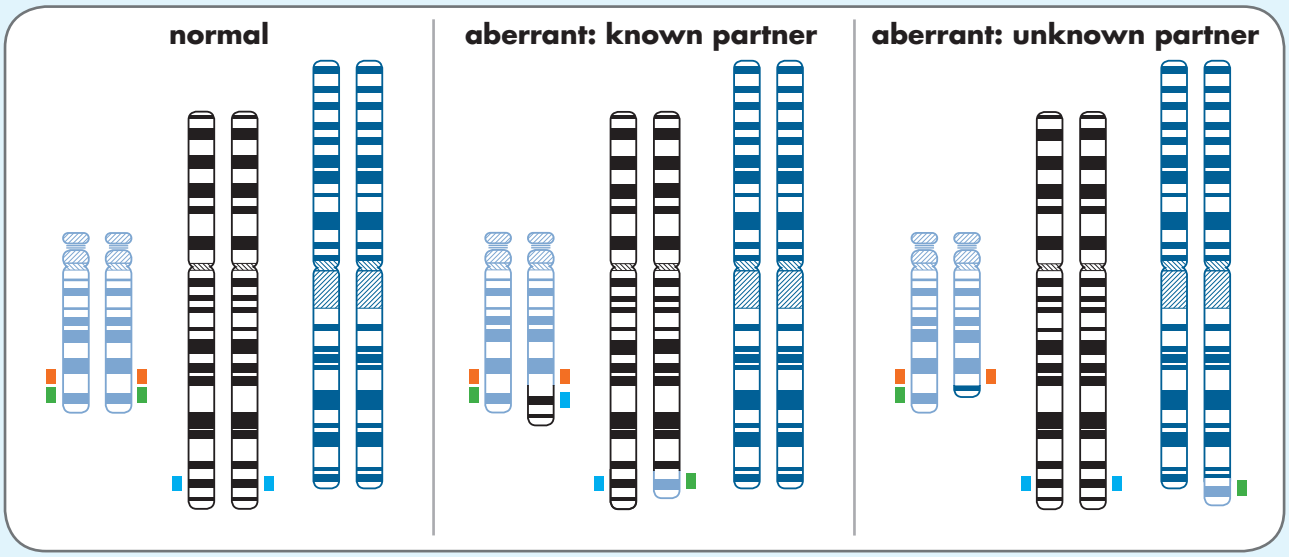
Other signal patterns than those described above may be observed in some abnormal samples. These unexpected signal patterns should be further investigated.

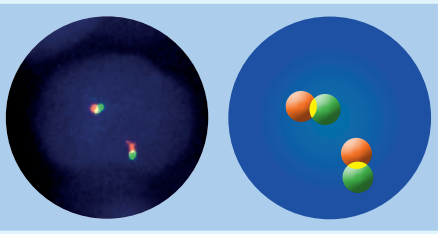
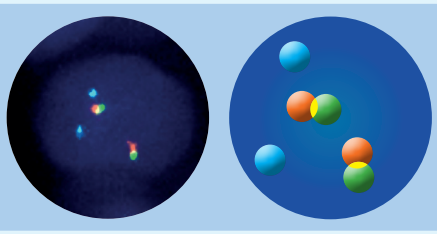
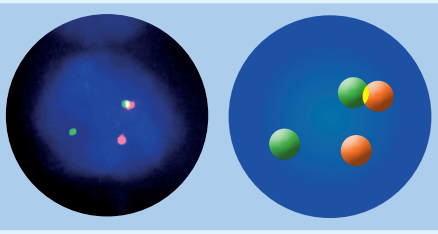
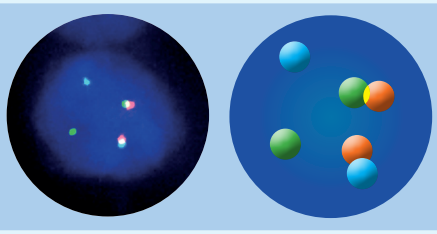
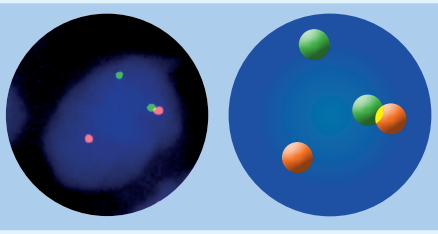
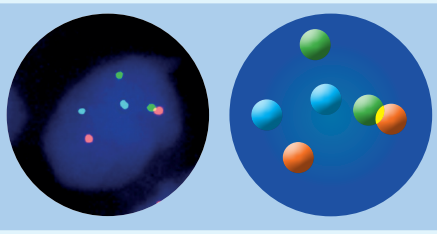
TriCheck™ Probe Design for Translocation

ZytoLight® FlexISH®

e.g. ZytoLight® SPEC FOXO1/PAX3 TriCheck™ Probe

These **TriCheck™ Probes** are designed for the detection and discrimination of translocations with known and unknown partners. The innovative probe design, consisting of three direct labeled probes (green, orange and blue), allows a fast and easy initial screening comparable to Dual Color Break Apart Probes by using a ZyGreen™/ZyOrange™ Dual Bandpass Filter Set. In nuclei showing break apart patterns, the usage of the ZyBlue™ Single Bandpass Filter Set allows a confirmation of the rearrangement and a discrimination between translocations with known and unknown partners.



	Green/Orange Dual Bandpass Filter	Merged (Green/Orange + Blue Filter)	
Normal			Two green/orange (or yellow) fusion signals, two blue signals.
Translocation, known partner			One separate green and orange signal, with one blue/orange co-localization.
Translocation, unknown partner			One separate green and orange signal, with NO blue/orange co-localization.

Rearrangement criteria: Distance between splitted signals (green and orange) ≥ 2 of the estimated signal diameter.

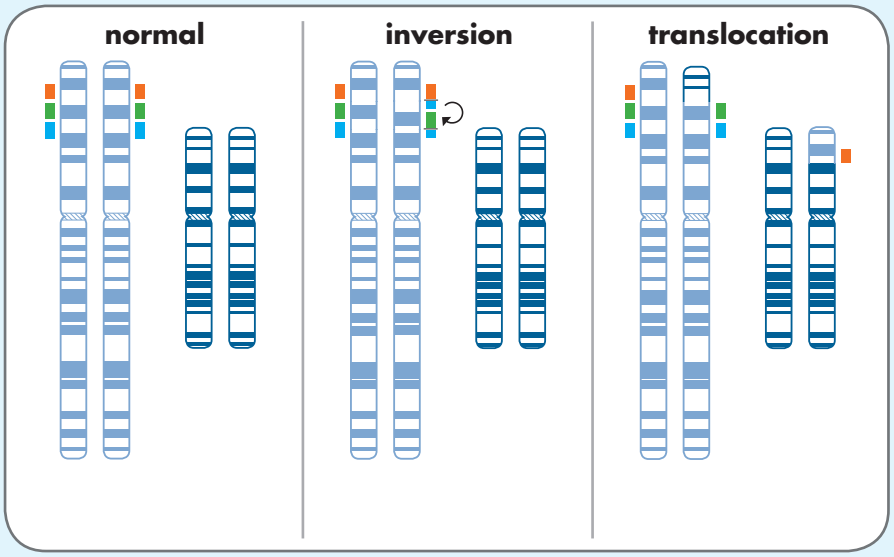
Other signal patterns than those described above may be observed in some abnormal samples. These unexpected signal patterns should be further investigated.

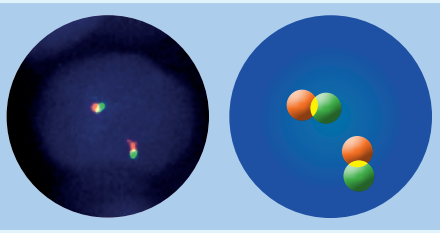
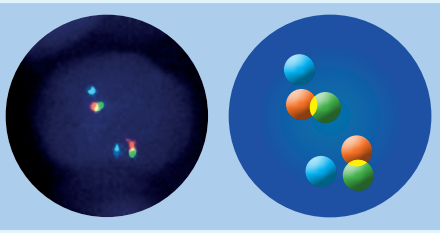
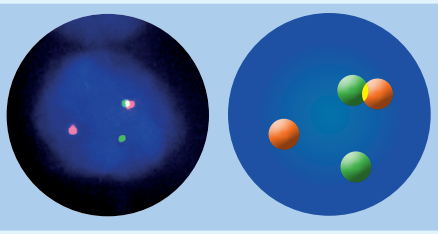
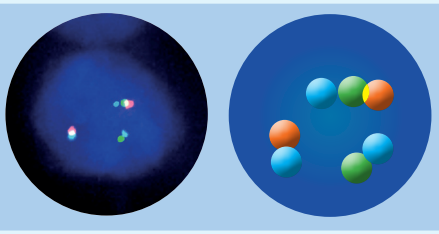
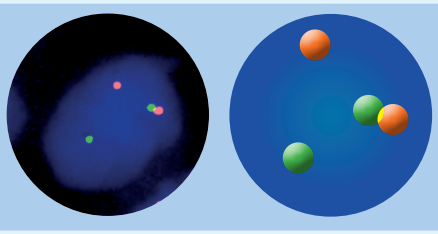
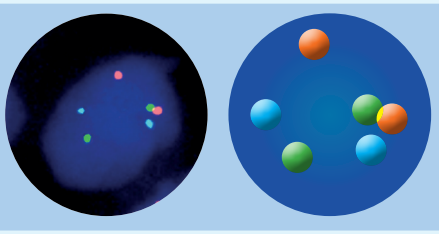
TriCheck™ Probe Design for Inversion

ZytoLight® FlexISH®

e.g. ZytoLight® SPEC ALK/EML4 TriCheck™ Probe

These **TriCheck™ Probes** are designed for the detection and discrimination of translocations and inversions. The innovative probe design, consisting of three direct labeled probes (green, orange and blue), allows a fast and easy initial screening comparable to Dual Color Break Apart Probes by using a ZyGreen™/ZyOrange™ Dual Bandpass Filter Set. In nuclei showing break apart patterns with even subtle signal separation, the usage of the ZyBlue™ Single Bandpass Filter Set allows a confirmation of the rearrangement and a discrimination between translocations and inversions. A patent for this probe design was granted in Germany, China, USA, and other European countries.



	Green/Orange Dual Bandpass Filter	Merged (Green/Orange + Blue Filter)	
Normal			**Two green/orange (or yellow) fusion signals, two blue signals.**
Inversion			**One separate green and orange signal, with one extra blue signal co-localized.**
Translocation			**One separate green and orange signal, with NO blue co-localization.**

Rearrangement criteria: Distance between splitted signals (green and orange) ≥ 1 of the estimated signal diameter.

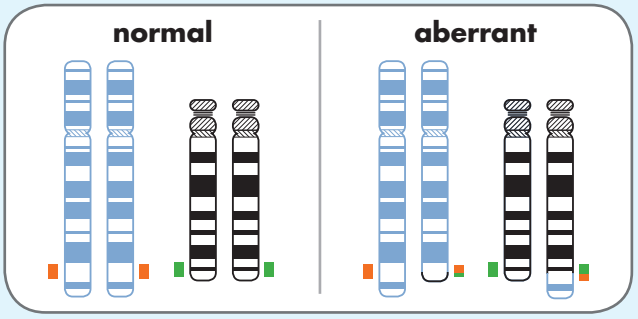
Other signal patterns than those described above may be observed in some abnormal samples. These unexpected signal patterns should be further investigated.

Dual Color Dual Fusion Probe Design

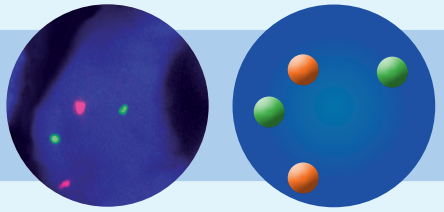
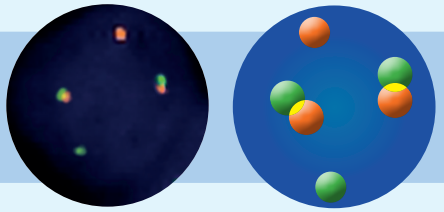
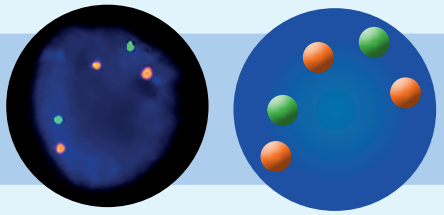
ZytoLight®

e.g. ZytoLight® SPEC MYC/IGH Dual Color Dual Fusion Probe

Dual Color Dual Fusion Probes are designed for the detection of specific fusions of two known fusion partners. **Dual Color Dual Fusion Probes** consist of two direct labeled probes (green and orange) spanning the breakpoint regions of both translocation partners.



Signal Pattern

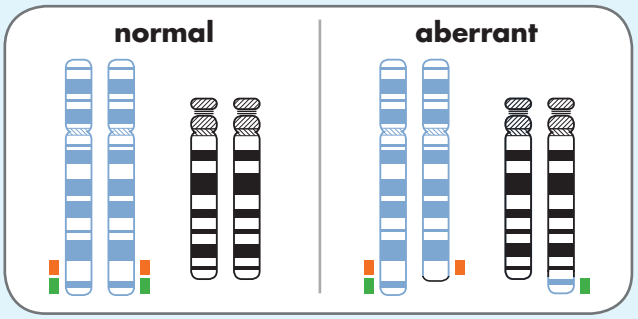
Normal		**Two single green** and **two single orange** signals.
Fusion		**Two green**/**orange** (or **yellow**) **fusion** signals, **one single green** and **one single orange** signal.
Fusion with an unknown partner or gene duplication or trisomy		**Two single green** signals and **three single orange** signals.

Dual Color Break Apart Probe Design

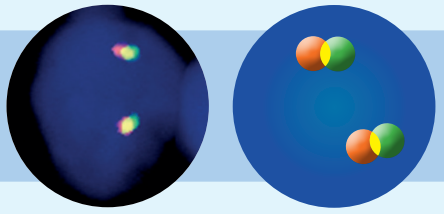
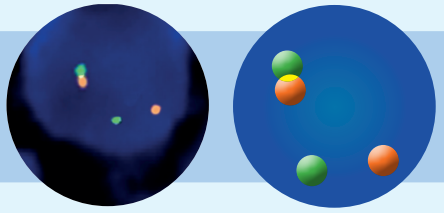
ZytoLight® ZytoMation®

e.g. ZytoLight® SPEC BCL2 Dual Color Break Apart Probe

Dual Color Break Apart Probes are designed for the detection of translocations involving multiple and/or unknown translocation partners where only the rearrangement of the targeted gene is of biological significance and not a specific type of fusion.
Dual Color Break Apart Probes consist of two direct labeled probes (green and orange) hybridizing distal and proximal to the gene breakpoint region.



Signal Pattern

Normal		**Two orange**/**green** (or **yellow**) **fusion** signals.
Translocation		**One green**/**orange** (or **yellow**) **fusion** signal, **one single green** and **a separate orange** signal.

Rearrangement criteria: Distance between splitted signals (green and orange) ≥ 2 of the estimated signal diameter.

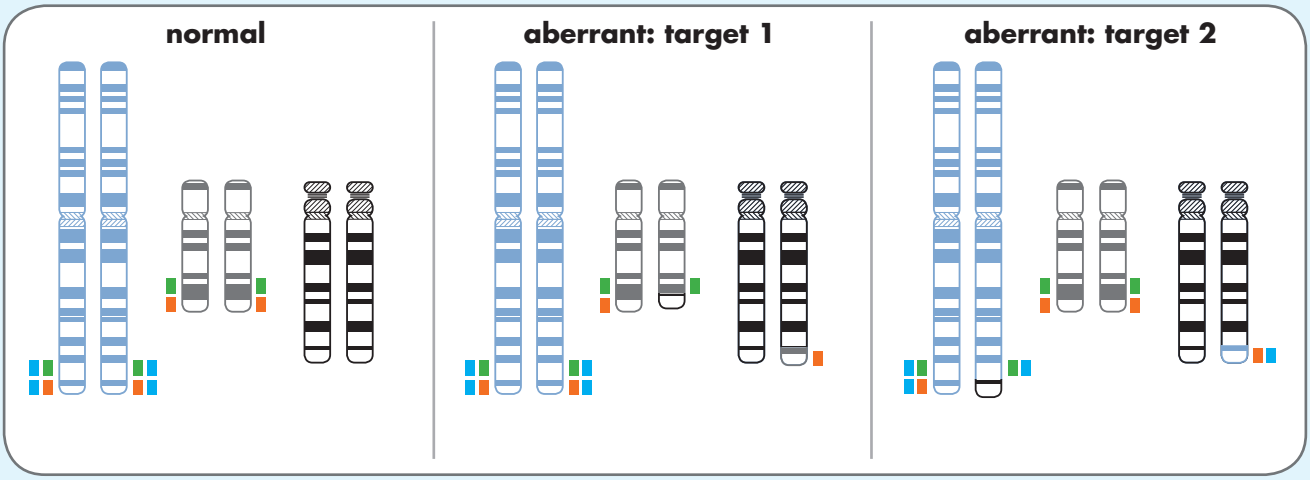
Other signal patterns than those described above may be observed in some abnormal samples. These unexpected signal patterns should be further investigated.

DistinguISH™ Probe Design

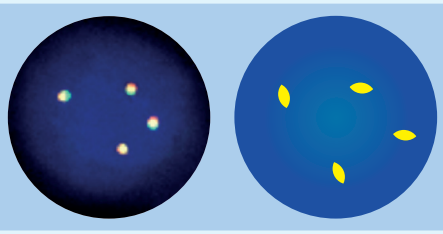
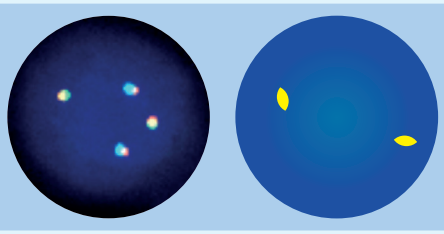
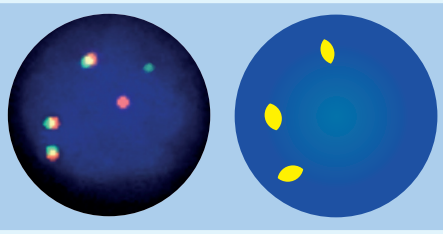
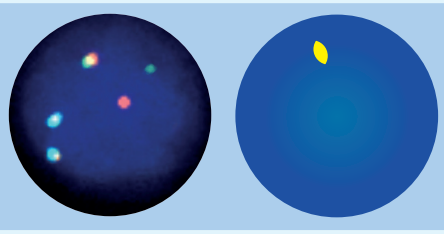
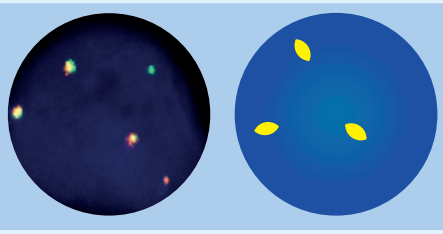
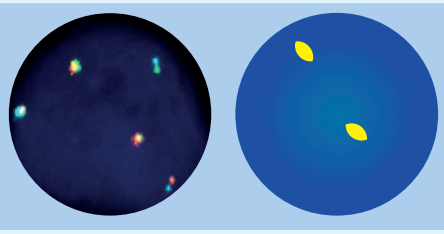
FlexISH®

e.g. FlexISH® BCL2/BCL6 DistinguISH™ Probe

DistinguISH™ Probes are designed to simultaneously detect two independent gene rearrangements. This innovative probe design enables the user to discriminate between rearrangements affecting two different gene loci in a single nucleus. Less patient material and evaluation time are thus needed, compared with running two FISH assays.
Using a ZyGreen™/ZyOrange™ Dual Bandpass Filter Set for initial screening allows the identification of aberrant nuclei. The subsequent use of a ZyBlue™ Single Bandpass Filter Set then indicates which gene locus is affected by the rearrangement. A patent for this probe design was granted in Germany, Japan, South Korea, and other European countries.



Signal Pattern

	Green/Orange Dual Bandpass Filter	Merged (Green/Orange + Blue Filter)	
Normal			**Two target 1** specific green/orange (or yellow) **fusion** signals and **two target 2** specific green/orange/blue **fusion** signals.
Aberrant target 1			**Separate green** and **orange** signal, with **NO blue co-localization**.
Aberrant target 2			**Separate green** and **orange** signal **co-localizing** with **blue** signals.

Rearrangement criteria: Distance between splitted signals (green and orange) ≥ 2 of the estimated signal diameter.

Other signal patterns than those described above may be observed in some abnormal samples. These unexpected signal patterns should be further investigated.

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For more product information please contact info@zytovision.com or your local dealer.

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ZYTOVISION
Molecular diagnostics simplified