

POSEIDON NEWSLETTER



UniStar – a Universal *In Situ* Hybridization Assay Designed for FISH and CISH on the Same Specimen

Chromogenic *In Situ* Hybridization (CISH) is increasingly emerging as a viable alternative to FISH and often selected as the method of choice for molecular pathologists for visualizing over-expression of genes involved in tumor development. CISH, like FISH, directly visualizes the number of gene copies present in the nucleus, and it produces a permanent record of the slide that can be interpreted with a regular light microscope in the context of the tumor histopathology.

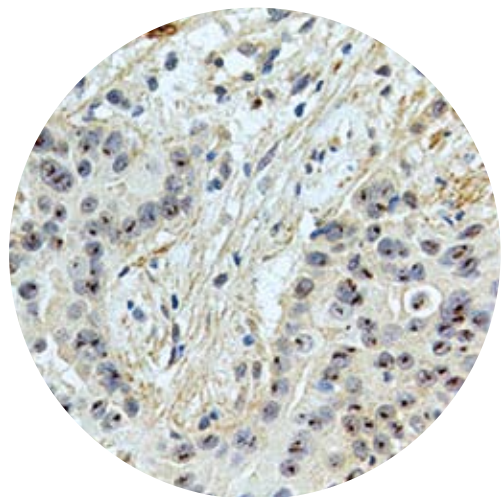


Image kindly provided by Dr. M. Gosens and Dr. E. Moerland, Stichting PAMM, Eindhoven, The Netherlands.

With the introduction of the UniStar product range, KREATECH is offering a novel assay based on its well established POSEIDON™ Repeat-Free™ DNA probes, enabling the clinician to consecutively perform FISH and CISH on the same sample. In a first step, the specific fluorescent-labeled DNA probes are hybridized to the sample enabling analysis under a fluorescence microscope. Subsequently, the fluorescent signal of the gene of interest can be converted into a chromogenic signal, which can then be analyzed with a regular bright field microscope.

The first UniStar assays introduced are designed for detecting Her2/Neu, EGFR, and the membrane bound receptor C-MET, respectively. Each of these kits include the corresponding Repeat Free™ DNA probes for copy number detection of the gene of interest, as well as a control probe for performing FISH in a dual-color assay. In addition, they include a specific detection module converting the signal of the critical probe into a chromogenic single-color signal via a colorimetric assay system.



CISH versus FISH – a brief comparison:

	CISH	FISH
Microscope	Bright-field	Fluorescence
Signal stability	Long-time storage	Fades over time
Simultaneous observation of tissue morphology	Good	Limited
Amount of training required	Medium	High
Overall cost	Medium	High
Slide Storage Temperature	Room temperature	4° C
Protection from light	Not Required	Required

Why CISH?

Chromogenic detection via bright field microscopy is the method of choice for many pathologists who do not have access to a fluorescence microscope, or who simply do not see the need to perform FISH, in particular as recent developments have made colorimetric detection equivalent to FISH in terms of accuracy. In addition, by using a bright field microscope, tissue morphology can be studied more easily, and signals can be archived for later analysis.

Assay set-up for FISH and CISH analysis with UniStar

Upon mounting of the formalin-fixed paraffin-embedded tissue section onto a microscope slide, a conventional FISH assay is performed by pretreating the slide to enable efficient hybridization of a fluorescent DNA probe. Subsequently, the signals can be analyzed with a fluorescent microscope followed by conversion of the FISH signal into a chromogenic signal followed by imaging using a conventional bright field microscope (A). Alternatively, the hybridized probes can directly be analyzed with CISH (B).

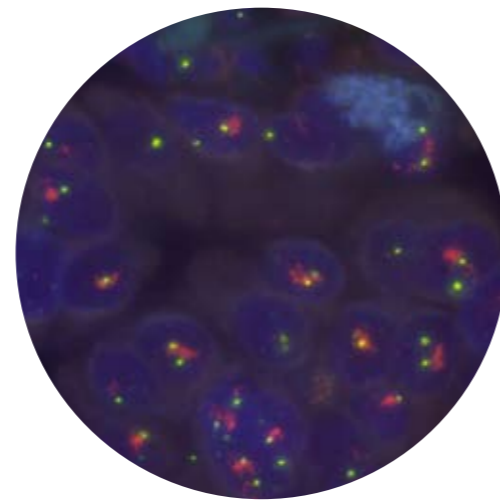
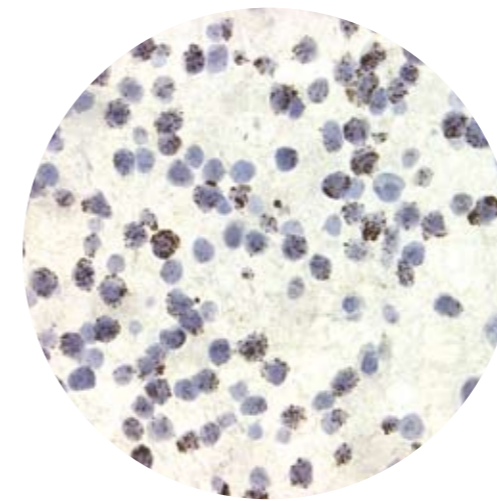
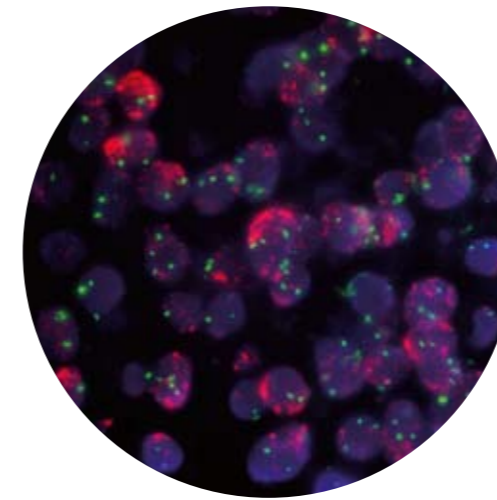


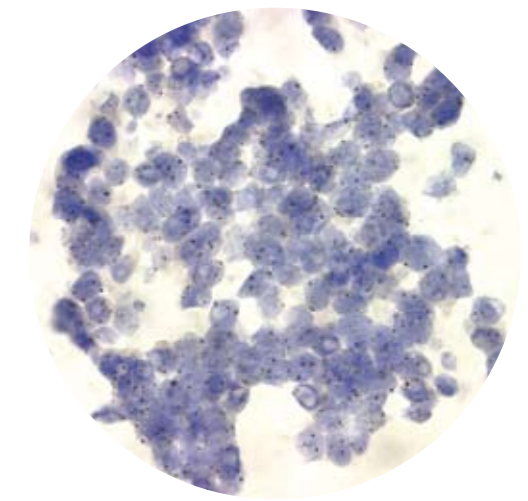
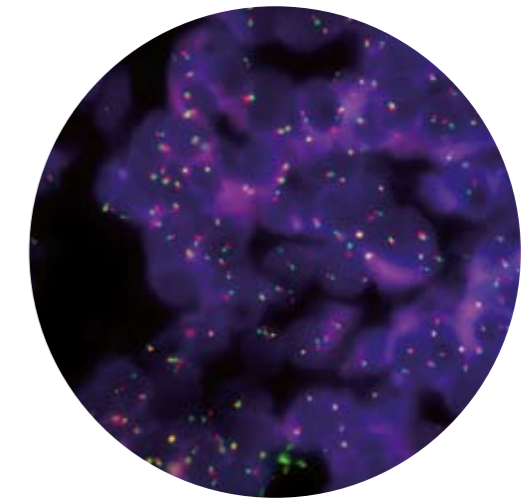
Image kindly provided by Dr. M. Gosens and Dr. E. Moerland, Stichting PAMM, Eindhoven, The Netherlands.

Fig. 1: Example of FISH and CISH performed consecutively on the same slide: breast cancer tissue hybridized with the Her-2/Neu / SE7 POSEIDON fluorescent probe followed by conversion of the red Her-2/Neu signal into a brownish chromogenic signal using the UniStar CISH Detection Kit (see front page).



EGFR images kindly provided by Dr. K. Beiske, Oslo University Hospital, Norway

Fig. 2: Glioblastoma specimen showing amplified EGFR. The slide was hybridized with the EGFR / SE7 POSEIDON fluorescent probe followed by conversion of the red EGFR signal into a colorimetric signal using the UniStar CISH Detection Kit.



EGFR images kindly provided by Dr. K. Beiske, Oslo University Hospital, Norway

Fig. 3: Colon carcinoma specimen showing a normal EGFR expression (non-amplified). The slide was hybridized in a sequential fashion as in Fig 2.



Tissue

Slide Pretreatment

Probe Hybridization



Assay set-up for FISH and CISH analysis with UniStar

For more information please visit our website: www.kreatech.com

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Ordering Information

Cat.#	Product	Description
KBI-50701	UniStar Her-2/Neu (17q12)	DNA probes specific for Her-2/Neu and SE 17, UniStar CISH Detection Kit
KBI-50702	UniStar EGFR (7p11)	DNA probes specific for EGFR and SE 7, UniStar CISH Detection Kit
KBI-50719	UniStar C-MET (7q31)	DNA probes specific for C-MET and SE 7, UniStar CISH Detection Kit
KBI-50001	UniStar CISH Detection Kit	UniStar CISH Detection Kit for the use with POSEIDON DNA Probes labeled in red
KBI-60007	POSEIDON Tissue Digestion Kit I	POSEIDON Tissue Digestion Kit I for pretreatment of paraffin-embedded tissue for subsequent analysis by CISH

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