BIOSYSTEMS

UniStar CISH Instruction Manual

For the use with UniStar Kits

KBI-50001; ERBB2 (KBI-50701); EGFR (KBI-50702); and MET (KBI-50719)

UniStar Kit components:

Component Description	Cat #	Cap Insert Color
Kreatech™ FISH probes (various)	KBI-xxxxx	Yellow (except for KBI-50001)
Wash Buffer III (10x)	LK-112	N.A
Blocking Buffer	LK-113	White
M-α-PB 550 Primary Antibody *Ready to Use*	LK-114	Red
G-α-M-HRP Secondary Antibody *Ready to Use*	LK-115	Blue
Substrate Buffer	LK-116	Black
HRP substrate (DAB)	LK-117	Purple

Slide pretreatment based on Kreatech Tissue Digestion Kit I (KBI-60007):

In case of heavily cross-linked paraffin-embedded tissue Kreatech Tissue Digestion Kit II (KBI-60004) is recommended.

1. Bake mounted slides for 2-16 hours at 56 °C.
2. De-paraffinize warm slides in xylene 2 x 5 minutes (min) at room temperature (RT).
3. Re-hydrate by soaking in 100%, 85% and 70% ethanol for 3 min each.
4. Wash with ultrapure water for 3 min at RT.
5. Place slides in Pretreatment Solution A (LK-110) at 96-98 °C for 15 min. Rinse twice with dH_2O for 2 min at RT.
6. Cover paraffin section with approximately 200 µl Pepsin Solution (LK-101) and incubate at RT for 5-50 min (time depending on tissue fixation and tissue type. E.g. most breast cancer tissue needs 5-15 min digestion; colon tissue 20-30 min).
7. Wash in ultrapure water for 1 min and in 2 x SSC (LK-104) for 5 min at RT.
8. Dehydrate slides by soaking in 70%, 85%, and 100% ethanol for 1 min each time.
   Air-dry. Proceed with the denaturation/ hybridization procedure.

Co-Denaturation using Kreatech™ FISH probes labeled with Platinum*Bright*™550:

Apply 10 µl of probe per 22 x 22 mm field. Cover with glass cover slip and seal with Fixogum or rubber cement. Denature sample and probe on a hot plate at 80 ±1 °C for 5 min. Continue with hybridization.

Note: Probes have been qualified on semi-automated hybridization-machines (e.g. ThermoBrite™).

Hybridization:

Incubate overnight at 37 ±1 °C in a humidified chamber.

Post-Hybridization Wash:

1. Remove rubber cement seal. Wash slides in Wash Buffer II (LK-103) for 2 min at RT to slide off cover slip.
2. Wash slides in Wash Buffer I (LK-102) for 2 min at 72 °C ±1 °C without agitation. Do not place more than 5 slides in the wash at one time. If more than 5 slides are to be washed, verify that the temperature on the wash solution is 72 °C ±1 °C. *Start timing the 2 min incubation when the last slide has been placed.*
3. Wash slides in Wash Buffer II for 1 min at RT without agitation.

A When carrying out FISH analysis first

4. Proceed immediately with the DAPI / antifade procedure to examine the results by fluorescence microscopy. After the examination proceed with the single color chromogenic detection procedure.

Note: Unlike conventional FISH protocols, do not de-hydrate the slides prior to application of DAPI counterstain, as over-dried slides might result in weak or absence of signals.

Alternatively, the DAPI / Antifade and fluorescence microscopy steps can be skipped. Instead, directly proceed with the single color chromogenic detection procedure **(section B)**.

DAPI / antifade procedure:
Apply 15 µl DAPI counterstain (LK-096) and apply glass cover slip. Proceed with microscopy.

Fluorescence microscopy:
For optimal visualization use a well maintained and regularly calibrated microscope equipped with a 100 W mercury lamp and a x 63 or x 100 Fluorescent objective. Triple band-pass filters (DAPI/FITC/Texas Red or DAPI/FITC/Rhodamine) are used to view multiple colours, single band-pass filters are used for individual colour visualization.

Suitable excitation and emission range for Kreatech™ fluorophores:
Platinum*Bright*™495 Ex 495 ±20 nm Em 525 ±30 nm
Platinum*Bright*™550 Ex 546 ±12 nm Em 580 ±30 nm

After examination by fluorescence microscopy to proceed with the DAPI /antifade washing procedure.

DAPI /antifade washing procedure:
For the preparation of the 1 x Wash Buffer III see preparation of working solutions below.

1. Wash the slides in 1 x Wash Buffer III for 2 min at RT.
2. Gently remove the coverslip
3. Wash the slides in 1 x Wash Buffer III for 2 x 2 min at RT.
4. Proceed with Section B Chromogenic detection procedure.

B When directly proceeding to CISH analysis

Preparation of working solutions:
- **1 x Wash Buffer III:** dilute 10 x Wash Buffer III (LK-112) 1: 10 with ultrapure water
- **Substrate:** for each slide add 4.5 µl HRP Substrate (DAB) *(LK-117, purple cap)* to 150 µl Substrate Buffer *(LK-116, black cap)*. **Prepare fresh just prior to use.**

Chromogenic detection procedure:
Note: Make sure that during the detection the tissue does not dry out!

1. Wash the slides in 1 x Wash Buffer III for 2 min at RT.
2. Apply 150 µl of Blocking Buffer *(LK-113, white cap)*.
3. Incubate the slides 10 min at RT.
4. **Wash the slides in 1 x Wash Buffer III for 2 x 2 min at RT.**
5. Apply 150 µl of M-α-PB550 Primary Antibody, RtU *(LK-114, red cap)*.
6. Incubate the slides 30 min at RT.
7. Wash the slides in 1 x Wash Buffer III for 2 x 2 min at RT.
8. Apply 150 µl of G-α-M-HRP Secondary Antibody, RtU *(LK-115, blue cap)*.
9. Incubate the slides 30 min at RT.
10. Wash the slides in 1 x Wash Buffer III for 2 x 2 min at RT.
11. Apply 150 µl of fresh prepared working solution HRP substrate (DAB) solution.
12. Incubate the slides 30 min at RT.
13. Wash the slides in 1 x Wash Buffer III for 2 min at RT.
14. Wash the slides in ultrapure water for 2 x 2 min at RT.
15. Counterstain the slides with Mayer's hematoxylin for 3-8 seconds.

Note: We strongly recommend a light counterstain in order to prevent masking of the chromogenic signal.

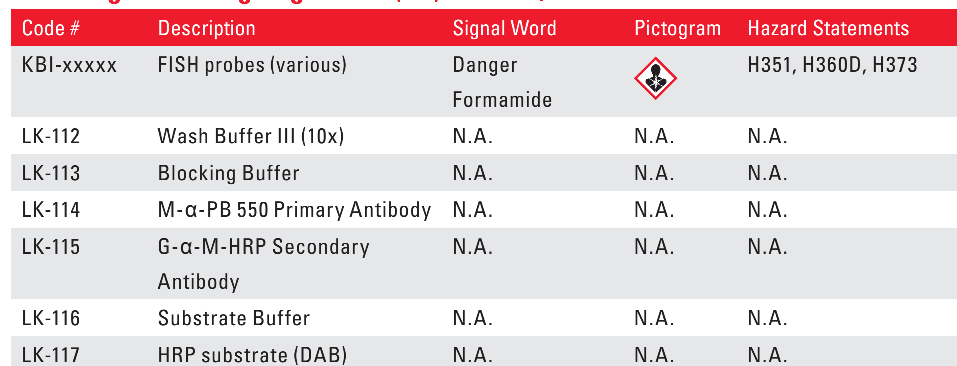
16. Wash the slides in ultrapure water for 2 x 2 min at RT.
17. De-hydrate the slides using 70% / 85% / 100% ethanol, 1 min each at RT.
18. Immerse the slides xylene for 2 x 2 min.
19. Embed the tissue under a proper coverslip using a xylene-based mounting medium.
20. Proceed with brightfield microscopy.

Brightfield microscopy:
Examine the hybridization results and the tissue morphology using a brightfield microscope (with a 40 x or 63 x objective).

Material required, but not supplied:
- Kreatech Tissue Digestion Kit I (KBI-60007)
- Kreatech Tissue Digestion Kit II (KBI-60004)
- Hot plate (80 °C)
- Incubator (37 °C)
- ThermoBrite™ (TS01/TS02) *optional*
- Water bath (96-98 °C / 72 °C)
- Xylene
- Ethanol
- Fixogum or rubber cement
- Mayer's Hematoxylin
- Xylene-base mounting medium
- Coplin jars (40-50 ml)
- Coverslips

Warnings and Precautions:
1. **For *in vitro* use only. For professional use only.** In case of emergencies check SDS sheets for safety information.
2. DNA probes and hybridization buffers contain formamide which is a teratogen; do not inhale or allow skin contact. Wear gloves and a lab coat when handling DNA probes and DAPI counterstain.
3. All materials should be disposed of according to your institution's guidelines for hospital waste disposal.

Labelling According Regulation (EC) No 1272/2008



Code #	Description	Signal Word	Pictogram	Hazard Statements
KBI-xxxxx	FISH probes (various)	Danger Formamide		H351, H360D, H373
LK-112	Wash Buffer III (10x)	N.A.	N.A.	N.A.
LK-113	Blocking Buffer	N.A.	N.A.	N.A.
LK-114	M-α-PB 550 Primary Antibody	N.A.	N.A.	N.A.
LK-115	G-α-M-HRP Secondary Antibody	N.A.	N.A.	N.A.
LK-116	Substrate Buffer	N.A.	N.A.	N.A.
LK-117	HRP substrate (DAB)	N.A.	N.A.	N.A.

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