KBI-40051 Kreatech™ PreimpScreen Blas

Instructions for Use

The PreimpScreen Blas probes are designed for the use on human polar body or blastomere cells. It consists of a 5-color, five probe mixture of DNA probe sequences homologous to specific regions on chromosomes 13, 18, 21, X, and Y. Each of the probes is directly labeled with one of Kreatech's ULS- fluorophores:

Critical region 1 **(dark red):**
The 13q14 region probe is direct-labeled with Platinum*Bright*™590.
Critical region 2 **(green):**
The 21q22 region probe is direct-labeled with Platinum*Bright*™495.
Critical region 3 **(light red):**
The Xp11 region probe is direct-labeled with Platinum*Bright*™547
Critical region 4 **(blue):**
The Yq11 probe is direct-labeled with Platinum*Bright*™415.
Critical region 5 **(dark blue):**
The **Satellite Enumeration 18** probe is direct-labeled with Platinum*Bright*™405.

Kreatech™ FISH probes do not contain Cot-1 DNA. Hybridization efficiency is therefore increased and background, due to unspecific binding, is highly reduced.

Specimen preparation:

Please prepare specimens according published protocols such as:
A laboratory guide to the mammalian embryo; Von David K. Gardner, Michelle Lane, Andrew John Watson. By Oxford University Press US, 2004.

Pretreatment:

Pretreat the slides to allow probe hybridization. Use published protocols. Alternatively the following protocol can be used:

1. Place slides in a Coplin jar containing PBS at room temperature (RT) for 3 min.
2. Dehydrate the slides in 70%, 85% and 100% ethanol for 3 min each.

Cell localization:

For whole embryos, possibly for binucleate or multinucleate blastomeres and not clear nucleus localization:

a. Apply 10 µl of DAPI/antifade to the target area.
b. Place a cover slip on top of the DAPI/antifade.
c. Localize the positions of nucleus/nuclei/nucleus fragments.
d. Take a picture under 10x objective or/and record the coordinates.
e. Soak the slides in 2 x SSC until the cover slip dislodges.
f. Dehydrate the slides in 70%, 85 % and 100% ethanol for 3 min each.

Continue with Pretreatment:

3. Prepare pepsin working solution by adding 0.5 ml of pepsin stock solution (10 mg/ml) to 49 ml H_2O and 0.5 ml 1 M HCl.
4. Pre-warm the pepsin solution (37 ±1 °C).
5. Immerse the slides in the pre-warmed pepsin solution for 20 min at 37 °C.
6. Rinse slides briefly in H_2O, followed by PBS.
7. Incubate slides in 1% Paraformaldehyde / PBS for 10 minutes at 4 °C.
8. Rinse slides briefly in PBS, then twice in H_2O at RT.
9. Let the remaining liquid pour down on paper tissue but do not let the slide dry.
10. Dehydrate the slide preparation 70%, 85% and 100% ethanol for 3 min each.
11. Transfer slides to a paper towel on the bench top or into slide rack and blot the edges of the slides on the paper towel to remove excess alcohol.
12. Air-dry at RT.

Denaturation:

1. Denature probe at 75 ±1 °C for 10 min.
2. Apply 3 µl of probe at a 10 mm round cover slip.
3. Invert slide and put it onto the cover slip. Put Parafilm on top marking a rectangular surrounding the cover slip.
4. Co-denature probe and sample for 90 seconds at 75 ±1 °C.

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

Hybridization:

Incubate for 2.5 - 4 hr at 37 ±1 °C in a humidified chamber.

Post-Hybridization Wash:

1. Remove Parafilm with fine forceps (use extreme caution to avoid moving the cover slip, as this could damage the nucleus).
2. Wash slides in 2 x SSC for 2 min at RT to slide off cover slip.
3. Wash in 0.7 x SSC / 0.3% Tween20 at 72 °C for 90 seconds.
4. Wash in 2 x SSC at RT for 2 min.
5. Dehydrate in 70%, 85% and 100% ethanol for 1 min each.
6. Air-dry at RT.
7. Mount in Antifade **(without DAPI in order to prevent difficulties to observe blue colors)**.

Microscopy:

Locate cells using a Triple-Filter (e.g. Chroma 61010 or 61000v2) and use narrow-band single filters specific for each color as indicated below.

Note: we recommend the specimen to be viewed or imaged in the sequence mentioned below, in order to minimize the extent of photo bleaching.
By following this sequence the more sensitive fluorophores are exposed to light first.

Fluorophore	**Ex/Em**	**Color**	**Recommended Filter**
Platinum*Bright*™405	410/455	Dark Blue	Blue
Platinum*Bright*™415	429/470	Blue	Aqua
Platinum*Bright*™495	495/517	Green	FITC
Platinum*Bright*™547	547/565	Light Red	Cy3
Platinum*Bright*™590	587/612	Dark Red	Texas Red

Note: certain fluorophores are visible through multiple filters.

				Filter		
Fluorophore	**Blue**	**Aqua**	**FITC**	**Cy3**	**Texas Red**	**Chroma 61010 Triple**
Platinum*Bright*™405	+					+
Platinum*Bright*™415	+	+				+
Platinum*Bright*™495			+			+
Platinum*Bright*™547				+	+	+
Platinum*Bright*™590				+		+

Procedural recommendations:

Temperature and buffer concentration (stringency) of hybridization and washing are important, as lower stringency can result in non-specific binding of the probe to other sequences, and higher stringency can result in a lack of signal. Incomplete denaturation of target DNA can result in lack of signal.

Recommendations for Fluorescence Microscopy:

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

Material required, but not supplied:

- 2 x SSC solution (LK-104B)
- PBS
- Pepsin
- Wash Buffer (0.7 x SSC / 0.3% Tween20)
- 1% Paraformaldehyde / PBS
- DAPI Counterstain (LK-095A)
- Antifade mounting medium (Counterstain Diluent LK-097B)
- Ethanol 100%, 85% and 70%
- Hot plate (with accurate temperature control up to 75 °C)
- Incubator with accurate temperature at 37 °C
- Water bath with accurate temperature between 37 °C and 75 °C
- Plastic or glass Coplin jars
- 10 mm round glass cover slip
- Parafilm
- Variable micropipettes (1 µl - 200 µl)
- Fluorescence microscope equipped with suitable filters (see Microscopy).

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

Pictogram 

Signal word: Danger

Hazard statement(s)

H351 Suspected of causing cancer.
H360D May damage the unborn child.
H373 May cause damage to organs (Blood) through prolonged or repeated exposure if swallowed.

Precautionary statement(s)
P201 Obtain special instructions before use.
P281 Use personal protective equipment as required.
P308 + P313 IF exposed or concerned: Get medical advice/ attention.

Supplemental Hazard Statements none

Restricted to professional users.

References:

1. Verlinsky Y, Cieslak J, Ivakuneuko V, et al. 1998. Preimplantation diagnosis of common aneuploidies by the first and second polar body FISH analysis. J Ass Reprod Genet. 15:284-288
2. Verlinsky Y, Cieslak J, Ivakuneuko V, et al. 1995. Pregnancies following pre-conception diagnosis of common aneuploidies by fluorescent *in-situ* hybridization. J Human Reprod. 10:1923-1927
3. Verlinsky and Kuliev. 1994. Preimplantation Diagnosis of Genetic Diseases. New York. Wiley Liss.

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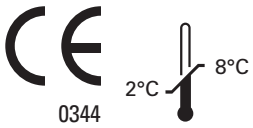

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