A Sysmex Group Company

CytoCell

Instructions For Use (IFU)

REF: CE-DES 500L, CE-DES 1000L

DAPI Antifade ES

CE IVD

PROFESSIONAL USE ONLY

ogt.com/IFU

Further information and other languages available at ogt.com/IFU

Intended Purpose
The CytoCell® DAPI Antifade ES counterstain solution is a qualitative, non-automated, accessory intended to aid visualisation of hybridised DNA fluorescence *in situ* hybridisation (FISH) probes using fluorescence microscopy.

Indications for Use
This device is an accessory intended to be used with CE marked CytoCell FISH Probes in accordance with their intended purpose and following instructions within the relevant IFU.

Limitations
This device is not intended for: use as a stand-alone diagnostic, use as a companion diagnostic, prenatal testing, population-based screening, near-patient testing, or self-testing.
This device has not been validated for sample types, disease types, or purposes outside of those stated in the intended purpose.
It is intended as an adjunct to other diagnostic laboratory tests and therapeutic action should not be initiated on the basis of the FISH result alone.
Reporting and interpretation of FISH results should be performed by suitably qualified staff, consistent with professional standards of practice, and should take into consideration other relevant test results, clinical and diagnostic information.
This device is intended for laboratory professional use only.
Failure to adhere to the protocol may affect the performance and lead to false positive/negative results.

Principles of the Test
Fluorescence *in situ* hybridisation (FISH) is a technique that allows DNA sequences to be detected on metaphase chromosomes or in interphase nuclei from fixed cytogenetic samples. The technique uses DNA probes that hybridise to entire chromosomes or single unique sequences, and serves as a powerful adjunct to G-banded cytogenetic analysis. This technique can now be applied as an essential investigative tool within prenatal, haematological and solid tumour chromosomal analysis. Target DNA, after fixation and denaturation, is available for annealing to a similarly denatured, fluorescently labelled DNA probe, which has a complementary sequence. Following hybridisation, unbound and non-specifically bound DNA probe is removed and the DNA is counterstained for visualisation. Fluorescence microscopy then allows the visualisation of the hybridised probe on the target material.

DAPI Antifade ES

Catalogue Number	Description	Volume
CE-DES 500L	DAPI Antifade ES (0.125µg/ml DAPI (4,6-diamidino-2-phenylindole) in glycerol-based mounting medium)	500µl
CE-DES 1000L	DAPI Antifade ES (0.125µg/ml DAPI (4,6-diamidino-2-phenylindole) in glycerol-based mounting medium)	1000µl

This DAPI Antifade ES kit contains only one of the two products noted above.

Materials Provided
DAPI Antifade ES counterstain solution: 500µl per vial or 1000µl per vial

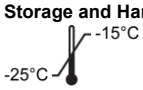
Warnings and Precautions

1. For *in vitro* diagnostic use. For laboratory professional use only.
2. Handle DAPI with care; wear gloves and a lab coat.
3. Do not use if the vial(s) are damaged, or the vial contents are compromised in any way.
4. Follow local disposal regulations for your location along with recommendations in the Safety Data Sheet to determine the safe disposal of this product. This also applies to damaged test kit contents.
5. Dispose of all used reagents and any other contaminated disposable materials following procedures for infectious or potentially infectious waste. It is the responsibility of each laboratory to handle solid and liquid waste according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.
6. Operators must be capable of distinguishing the colours red, blue, and green.
7. Failure to adhere to the outlined protocol and reagents may affect the performance and lead to false positive/negative results.
8. The probe should not be diluted or mixed with other probes.
9. Failure to use 10µl of probe during the pre-denaturation stage of the protocol may affect the performance and lead to false positive/negative results.
10. All products should be validated before use.
11. Internal controls should be carried out by using unaffected cell populations in testing samples.

Temperature Definitions

- -20°C / Frozen / In the Freezer: -25°C to -15°C
- 37°C: +37°C ± 1°C
- 72°C: +72°C ± 1°C
- 75°C: +75°C ± 1°C
- Room Temperature (RT): +15°C to +25°C

Storage and Handling



The kit should be stored between -25ºC to -15ºC in a freezer until the expiry date indicated on the kit label. The counterstain vial must be stored in the dark.

The DAPI Antifade ES counterstain remains stable throughout the freeze-thaw cycles experienced during normal use (where one cycle constitutes the vial's removal from and replacement into the freezer) - 50 cycles for the 500µl (50 tests) vial of DAPI Antifade ES, and 100 cycles for the 1000µl (100 tests) vial of DAPI Antifade ES . Exposure to light should be minimised and avoided wherever possible. Store components in a light proof container. Components used and stored under conditions other than those stated on the labelling may not perform as expected and may adversely affect the assay results. All efforts must be made to limit exposure to light and temperature changes.

Equipment, Materials, and Reagents Necessary but not Supplied
Calibrated equipment must be used:
1. Variable volume micropipettes and tips range 1µl - 200µl.
2. Consult the relevant CE marked CytoCell FISH Probe Kit IFU for any additional equipment, materials, and reagents necessary but not supplied.

Fluorescence Microscope Recommendation
Consult the relevant CE marked CytoCell FISH Probe Kit IFU for the appropriate microscope filter(s) to use.
Check the fluorescence microscope before use to ensure it is operating correctly. Use immersion oil that is suitable for fluorescence microscopy and formulated for low auto fluorescence. Avoid mixing DAPI antifade with microscope immersion oil as this will obscure signals. Follow manufacturers' recommendations in regards to the life of the lamp and the age of the filters.

Sample Preparation
Consult the relevant CE marked CytoCell FISH Probe Kit IFU for information regarding sample preparation.

DAPI Protocol
(Note: Ensure that exposure of the probe and counterstain to laboratory lights is limited at all times).

1. Consult the relevant CE marked CytoCell FISH Probe Kit IFU for the full FISH protocol.
2. Remove the DAPI from the freezer and allow it to warm to RT.
3. After removing a slide from the post hybridisation washes:
4. Drain the slide and apply 10-15µl of DAPI antifade onto each sample (specific volume depends on the CytoCell FISH Probe used - see step 1).
5. Cover with a coverslip, remove any bubbles and allow the colour to develop in the dark for 10 minutes.
6. View with a fluorescence microscope (see **Fluorescence Microscope Recommendation**).

Procedural Recommendations
1. Baking or ageing of slides may reduce signal fluorescence.
2. Hybridisation conditions may be adversely affected by the use of reagents other than those provided or recommended by Cytocell Ltd.
3. Use a calibrated thermometer for measuring temperatures of solutions, waterbaths and incubators as these temperatures are critical for optimum product performance.
4. The wash concentrations, pH and temperatures are important as low stringency can result in non-specific binding of the probe and too high stringency can result in a lack of signal.
5. Incomplete denaturation can result in lack of signal and over denaturation can also result in non-specific binding.
6. Over hybridisation can result in additional or unexpected signals.

7. Users should optimise the protocol for their own samples prior to using the test for diagnostic purposes.
8. Suboptimal conditions may result in non-specific binding that may be misinterpreted as a probe signal.

Interpretation of Results
Consult the relevant CE marked CytoCell FISH Probe Kit IFU for information regarding analysis guidelines and interpretation of results.

Expected Results
Consult the relevant CE marked CytoCell FISH Probe Kit IFU for information regarding expected results.

Known Relevant Interferences / Interfering Substances
Consult the relevant CE marked CytoCell FISH Probe Kit IFU for information regarding interferences / interfering substances.

Known Cross-Reactivity
Consult the relevant CE marked CytoCell FISH Probe Kit IFU for information regarding cross-reactivity.

Serious Incident Reporting
For a patient/user/third party in the European Union and in countries with identical regulatory regime (Regulation (EU) 2017/746 on In vitro Diagnostic Medical Devices); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the Manufacturer and to your National Competent Authority.
For serious incidents in other countries, please report it to the Manufacturer and, if applicable, to your National Competent Authority.
Manufacturer vigilance contact: vigilance@ogt.com
For EU National Competent Authorities, a list of vigilance contact points can be found at: https://ec.europa.eu/health/md_sector/contact_en

Specific Performance Characteristics
Not Applicable for DAPI Antifade ES counterstain solution.

Additional Information
For additional product information please contact the CytoCell Technical Support Department.
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Symbols Glossary

EN ISO 15223-1:2021 - "Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements" (© International Organization for Standardization)		
Symbol	**Title**	**Reference Number(s)**
	en: Manufacturer	5.1.1
EC REP	**en:** Authorized representative in the European Community/European Union	5.1.2
	en: Use-by date	5.1.4
LOT	**en**: Batch code	5.1.5
REF	**en:** Catalogue number	5.1.6
	en: Keep away from sunlight	5.3.2
	en: Temperature limit	5.3.7
	en: Consult instructions for use	5.4.3
ogt.com/IFU	**en:** Consult electronic instructions for use	5.4.3
IVD	**en:** *In vitro* diagnostic medical device	5.5.1
Σ	**en:** Contains sufficient for <n> tests	5.5.5
UDI	**en:** Unique Device Identifier	5.7.10

EDMA symbols for IVD reagents and components, October 2009 revision		
Symbol	**Title**	**Reference Number(s)**
CONT	**en:** Contents (or contains)	N/A

Patents and Trademarks
CytoCell is a registered trademark of Cytocell Limited.

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EC REP

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IFU Version History
V006 2022-05-26: New IFU for Regulation (EU) 2017/746
V007 2025-08-29: Removal of UKCA mark
V008 2025-09-09: Update to EU authorised representative address. Removal of EU authorised representative telephone number. Removal of OGT fax number.