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CytoCell

Instructions For Use (IFU)
REF: CE-LPA 001-S / CE-LPA 001 / CE-LPA 001-30 / CE-LPA 001-50

Prenatal Enumeration Probe Kit

CE IVD
2797

PROFESSIONAL USE ONLY

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Intended Purpose

The CytoCell® Prenatal Enumeration Probe Kit is a qualitative, non-automated, fluorescence *in situ* hybridisation (FISH) test used to detect the chromosome Xp11.1-q11.1, Yp11.1-q11.1 and 18p11.1-q11.1 alpha centromeric regions, and the chromosome 13q14.2 and 21q22.1 regions in Carnoy's solution (3:1 methanol/acetic acid) fixed cells derived from amniotic fluid samples, in enumerating chromosomes X, Y, 13, 18 and 21 in high risk pregnancies where Turner, Patau, Edwards or Down syndrome are suspected.

Indications for Use

This device is designed as an adjunct to other clinical and laboratory tests in recognised diagnostic and clinical care pathways, such as ultrasound screening and biochemical testing, where knowledge of the copy number status of the chromosome Xp11.1-q11.1 and 18p11.1-q11.1 alpha centromeric regions, the chromosome 13q14.2 and 21q22.1 regions would be important for patient management.

Limitations

This device is designed to detect chromosomal material which includes the centromeric regions covered by the green, orange and aqua clones in the Prenatal X, Y and 18 Enumeration Probe component, for chromosomes X, Y and 18 respectively. Genomic gains or losses outside these regions, or partial losses or gains of these regions may not be detected with this device.
This device is also designed to detect chromosomal material which includes the chromosome 13q14.2 and 21q22.1 regions covered by the green and orange clones in the Prenatal 13 and 21 Enumeration Probe component. Genomic gains or losses outside these regions, or partial losses or gains of these regions may not be detected with this product.
This device is not intended for: use as a stand-alone diagnostic, companion diagnostic, 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. This device has not been validated for use in detecting gains of chromosome X and/or Y.
This device 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

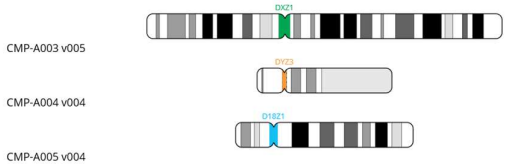
Fluorescence *In Situ* Hybridisation (FISH) is a technique that allows DNA sequences to be detected on metaphase chromosomes or in interphase nuclei from fixed cytogenetic samples. The technique uses DNA probes that hybridise to entire chromosomes or single unique sequences, and serves as a powerful adjunct to classic cytogenetics. Recent developments have meant that this valuable technique can now be applied as an essential diagnostic tool in prenatal, haematological and pathological 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.

Probe Information

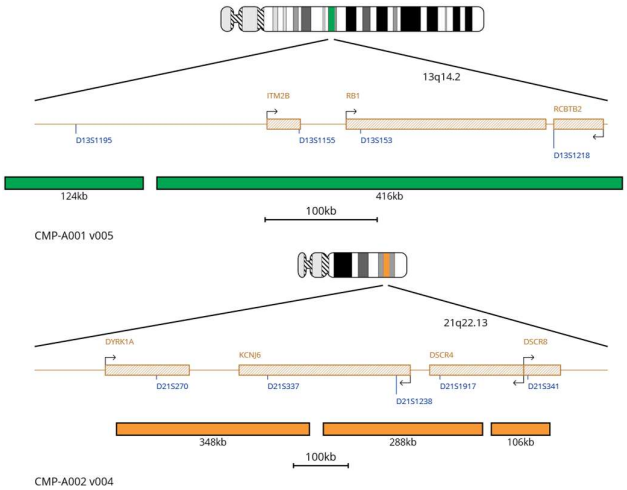
Molecular cytogenetic analysis plays a pivotal role in the prenatal evaluation of high-risk pregnancies, enabling the accurate identification of chromosomal abnormalities and the assessment of recurrence risk. The most common chromosomal abnormalities are aneuploidies, defined as an abnormal chromosome copy number, with affected individuals typically presenting with trisomy (three copies of a chromosome) or monosomy (single copy of a chromosome).[1] Well-known, clinically significant aneuploidies include trisomies 13, 18, and 21, as well as monosomy X. These abnormalities represent major contributors to developmental disorders and adverse reproductive outcomes and are among the most common indications for invasive prenatal diagnosis.[2]
Trisomy 21 (Down syndrome), the most common viable autosomal trisomy, is characterised by cognitive impairment, congenital cardiac anomalies, and multisystem morbidity, and remains a key indication for prenatal genetic testing.[3,4]
Trisomy 13 (Patau syndrome) is characterised by brain malformations (holoprosencephaly), facial dysmorphism, ocular anomalies, postaxial polydactyly, visceral malformations (cardiopathy) and severe psychomotor retardation.[5]
Trisomy 18 (Edwards syndrome) is associated with severe congenital malformations, high perinatal mortality, and well-defined ultrasonographic phenotypes, underscoring the necessity for accurate prenatal detection and genetic confirmation.[6,7] Monosomy X (Turner syndrome), also referred to as congenital ovarian hypoplasia syndrome, is the most common sex chromosomal abnormality in females and results from complete or partial absence of one X chromosome.[8]
Prenatal diagnosis is offered in high-risk pregnancies at increased risk of chromosomal abnormalities such as trisomy 13, 18, 21, and monosomy X. FISH is a validated molecular cytogenetic method for rapid detection and enumeration of chromosome copy number in interphase nuclei. FISH enables targeted detection of common aneuploidies and provides rapid results to support clinical decision-making and is widely used in modern aneuploidy diagnostics, particularly where prompt confirmation of chromosome copy number status is required.[9,10,11]

Probe Specification

LPA002 Prenatal X, Y and 18 Enumeration Probe
X centromere, Xp11.1 – q11.1 (DXZ1) Green
Y centromere, Yp11.1 – q11.1 (DYZ3) Orange
18 centromere, 18p11.1 – q11.1 (D18Z1) Aqua



LPA003 Prenatal 13 and 21 Enumeration Probe
13 unique sequence, 13q14.2 Green
21 unique sequence, 21q22.13 Orange



The kit contains two probe components for two separate hybridisations.

LPA002
The probe component LPA002 is a mixture of green, orange and aqua directly labelled fluorescent DNA probes:

- The green DXZ1 probe directly labels the alpha satellite DNA sequences at the DXZ1 region of the X chromosome.
- The orange DYZ3 probe directly labels the alpha satellite DNA sequences at the DYZ3 region of the Y chromosome.
- The aqua D18Z1 probe directly labels the alpha satellite DNA sequences at the D18Z1 region of chromosome 18.

LPA003
The probe component LPA003 is a mixture of green and orange directly labelled fluorescent DNA probes containing unique sequences:

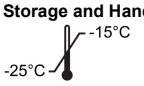
- The green probe mix contains a 124kb probe and a 416kb probe at 13q14.2 that spans the ITM2B, RB1 and RCBTB2 genes, including markers D13S1195, D13S1155, D13S153, and D13S1218.
- The orange probe mix contains a 348kb probe, 288kb probe and 106kb probe at 21q22.13 that spans the DYRK1A, KCNJ6, DSCR4 and DSCR8 genes, including markers D21S270, D21S337, D21S1238, D21S1917 and D21S341.

Materials Provided
Probe: 50µl per vial in 5 test kit, 100µl per vial in 10 test kit, 300µl per vial in 30 test kit, 500µl per vial in 50 test kit.
The probe components are provided premixed in hybridisation solution (<65% formamide; <20mg dextran sulfate; <10% of 20x saline-sodium citrate (SSC)) and are ready to use.

Counterstain: 150µl per vial in 5 test kit, 500µl per vial in 10 test kit, 1000µl per vial in 30 test kit, or 1200µl per vial in 50 test kit.
The counterstain is DAPI Antifade ES (0.125µg/ml DAPI (4,6-diamidino-2-phenylindole) in glycerol-based mounting medium).

Warnings and Precautions
1. For *in vitro* diagnostic use. For laboratory professional use only.
2. Operators must ensure that the correct and most current Instructions for Use are consulted and followed at all times.
3. Probe mixtures contain formamide, which is a teratogen; do not breathe fumes or allow skin contact. Handle with care; wear gloves and a lab coat.
4. Handle DAPI with care; wear gloves and a lab coat.
5. Do not use if the vial(s) are damaged, or the vial contents are compromised in any way.
6. 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.
7. 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.
8. Operators must be capable of distinguishing the colours aqua, orange, and green.
9. Failure to adhere to the outlined protocol, equipment and reagents may affect the performance and lead to false positive/negative results.
10. The probe should not be diluted or mixed with other probes.
11. 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.
12. All products should be validated before use.
13. Internal controls should be carried out by using unaffected cell populations in testing samples.
14. Precautions should be taken to prevent contamination between the vials containing LPA002 and LPA003.

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 probe and counterstain vials must be stored in the dark.


The FISH probe, DAPI Antifade ES counterstain and Hybridisation Solution remain stable throughout the freeze-thaw cycles experienced during normal use (where one cycle constitutes the vial's removal from and replacement into the freezer). **FISH probes:** 5 cycles for the 5 test kit, 10 cycles for the 10 test kit, 30 cycles for 30 test kit, 50 cycles for the 50 test kit. **Counterstain:** 10 cycles for the 5 test kit, 20 cycles for the 10 test kit, 60 cycles for the 30 test kit, and 100 cycles for the 50 test kit. Exposure to light should be minimised and avoided wherever possible. Store components in the light proof container provided. 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.

Equipment and Materials Necessary but not Supplied
Calibrated equipment must be used:
1. Hotplate (with a solid plate and accurate temperature control up to 80ºC)
2. Calibrated variable volume micropipettes and tips range 1µl - 200µl
3. Water bath with accurate temperature control at 37ºC and 72ºC
4. Microcentrifuge tubes (0.5ml)
5. Fluorescence microscope (Please see Fluorescence Microscope Recommendation section)
6. Phase contrast microscope
7. Clean plastic, ceramic or heat-resistant glass Coplin jars
8. Forceps
9. Calibrated pH meter (or pH indicator strips capable of measuring pH 6.5 – 8.0)
10. Humidified container
11. Fluorescence grade microscope lens immersion oil
12. Bench top centrifuge
13. Microscope slides
14. 24x24mm coverslips
15. Timer
16. 37ºC incubator
17. Rubber solution glue
18. Vortex mixer
19. Graduated cylinders
20. Magnetic stirrer
21. Calibrated thermometer

Optional Equipment not Supplied
1. Cytogenetic drying chamber

Reagents Needed but not Supplied
1. 20x saline-sodium citrate (SSC) Solution
2. 100% Ethanol
3. Tween-20
4. 1M Sodium hydroxide (NaOH)
5. 1M Hydrochloric acid (HCl)
6. Purified water

Fluorescence Microscope Recommendation
Use a 100-watt mercury lamp or equivalent and oil immersion plan apochromat objectives 60/63x or 100x for optimal visualisation. The fluorophores used in this probe kit will excite and emit at the following wavelengths:

Fluorophore	$Excitation_{max}$ [nm]	$Emission_{max}$ [nm]
Aqua	418	467
Green	495	521
Orange	551	572

Ensure appropriate excitation and emission filters that cover the wavelengths listed above are fitted to the microscope.

The triple bandpass filter DAPI/FITC/TRITC is optimal for viewing the green and orange fluorophores as well as counterstain simultaneously. The aqua fluorophore has specificity to the Aqua and DEAC spectrum (single bandpass Aqua or DEAC filter is required).

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
The kit is designed for use on Carnoy's solution (3:1 methanol/acetic acid) fixed cells derived from amniotic fluid samples, in enumerating chromosomes X, Y, 13, 18 and 21 in high-risk pregnancies where Turner, Patau, Edwards or Down syndrome are suspected, that are prepared according to the laboratory or institution guidelines. Amniotic fluid sample collection should be performed according to the laboratory or institution guidelines. Samples that appear bloody or brown should not be used, since they may contain maternal blood and may lead to false results. Prepare air dried samples on microscope slides according to standard cytogenetic procedures. The AGT *Cytogenetics Laboratory Manual* contains recommendations for specimen collection, culturing, harvesting and for slide making.[12]

Solution Preparation
Ethanol Solutions
Dilute 100% ethanol with purified water using the following ratios and mix thoroughly:
- 70% Ethanol - 7 parts 100% ethanol to 3 parts purified water
- 85% Ethanol - 8.5 parts 100% ethanol to 1.5 parts purified water

Store the solutions for up to 6 months at room temperature in an airtight container.

2xSSC Solution
Dilute 1 part 20xSSC Solution with 9 parts purified water and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

0.4xSSC Solution
Dilute 1 part 20xSSC Solution with 49 parts purified water and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

2xSSC, 0.05% Tween-20 Solution
Dilute 1 part 20xSSC Solution with 9 parts purified water. Add 5µl of Tween-20 per 10ml and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

FISH Protocol
(Note: Please ensure that exposure of the probe to laboratory lights is limited at all times)

Slide Preparation
1. Spot the cell sample onto a glass microscope slide. Allow to dry. (**Optional, if using a cytogenetic drying chamber**: The chamber should be operated at approximately 25°C and 50% humidity for optimal cell sample spotting. If a cytogenetic drying chamber is not available, use a fume hood as an alternative).
2. Immerse the slide in 2xSSC for 2 minutes at room temperature (RT) without agitation.
3. Dehydrate in an ethanol series (70%, 85% and 100%), each for 2 minutes at RT.
4. Allow to dry.

Pre-Denaturation
5. Remove the probe from the freezer and allow it to warm to RT. Briefly centrifuge tubes before use.
6. Ensure that the probe solution is uniformly mixed with a pipette.
7. Remove 10µl of probe per test, and transfer it to a microcentrifuge tube. Quickly return the remaining probe to the freezer.
8. Place the probe and the sample slide to prewarm on a 37ºC (+/- 1ºC) hotplate for 5 minutes.
9. Spot 10µl of probe mixture onto the cell sample and carefully apply a coverslip. Seal with rubber solution glue and allow the glue to dry completely.

Denaturation
10. Denature the sample and probe simultaneously by heating the slide on a hotplate at 75ºC (+/- 1ºC) for 2 minutes.

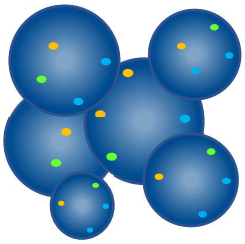
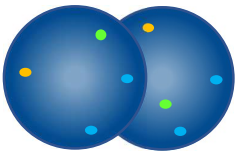
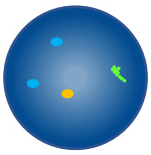
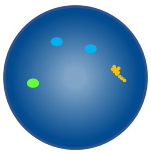
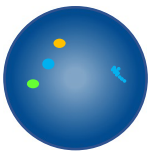
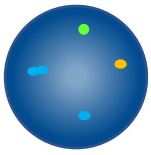
Hybridisation
11. Place the slide in a humid, lightproof container at 37ºC (+/- 1ºC) overnight.

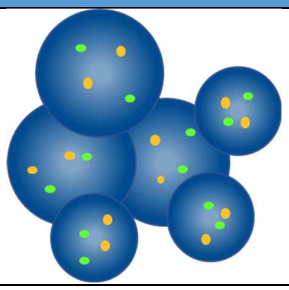
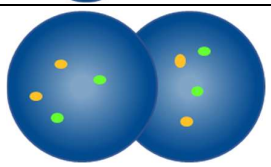
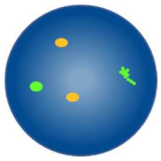
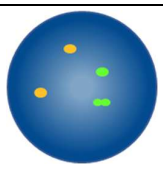
Post-Hybridisation Washes
12. Remove the DAPI from the freezer and allow it to warm to RT.
13. Remove the coverslip and all traces of glue carefully.
14. Immerse the slide in 0.4xSSC (pH 7.0) at 72ºC (+/- 1ºC) for 2 minutes without agitation.
15. Drain the slide and immerse it in 2xSSC, 0.05% Tween-20 at RT (pH 7.0) for 30 seconds without agitation.
16. Drain the slide and apply 10µl of DAPI antifade onto each sample.
17. Cover with a coverslip, remove any bubbles and allow the colour to develop in the dark for 10 minutes.
18. 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, water baths 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
Assessing Slide Quality
The slide should <u>not</u> be analysed if:
- Signals are too weak to analyse in single filters - in order to proceed with analysis, signals should appear bright, distinct and easily evaluable
- There are high numbers of clumped/overlapping cells obstructing the analysis
- >50% of the cells are not hybridised
- There is excess of fluorescent particles between cells and/or a fluorescent haze that interferes with the signals - in optimal slides the background should appear dark or black and clean
- Cell nucleus borders cannot be distinguished and are not intact

Analysis Guidelines
- Two analysts should analyse and interpret each sample. Any discrepancies should be resolved by assessment by a third analyst
- Each analyst should be suitably qualified according to recognised national standards
- Each analyst should independently score sufficient nuclei from each sample so that combined analysts' scores meet minimal criteria as specified by institutional, regional or national guidelines. The first analyst should start the analysis from the left side of the slide and the second analyst from the right
- Each analyst should document their results in separate sheets
- Analyse only intact nuclei, not overlapped or crowded nuclei or nuclei covered by cytoplasmic debris or high degree of autofluorescence
- Avoid areas where there is excess of cytoplasmic debris or non-specific hybridisation
- Signal intensity may vary, even with a single nucleus. In such cases, use single filters and/or adjust the focal plane
- In suboptimal conditions signals may appear diffuse. If two signals of the same colour touch each other, or the distance between them is no greater than two signal widths, or when there is a faint strand connecting the two signals, count as one signal
- If in doubt about whether a cell is analysable or not, then do not analyse it

Analysis Guidelines – LPA002	
	Do not count – nuclei are too close together to determine boundaries
	Do not count overlapping nuclei – all areas of both nuclei are not visible
	Count as one green signal, one orange signal and two aqua signals – the green signal is diffuse
	Count as one green signal, one orange signal and two aqua signals – the orange signal is diffuse
	Count as one green signal, one orange signal and two aqua signals – one of the two aqua signals is diffuse
	Count as one green signal, one orange signal and two aqua signals – the gap in the aqua signal is less than two probe widths

Analysis Guidelines - LPA003	
	Do not count – nuclei are too close together to determine boundaries
	Do not count overlapping nuclei – all areas of both nuclei are not visible
	Count as two orange and two green signals – one of the two green signals is diffuse
	Count as two orange and two green signals – the gap in one green signal is less than two signal widths

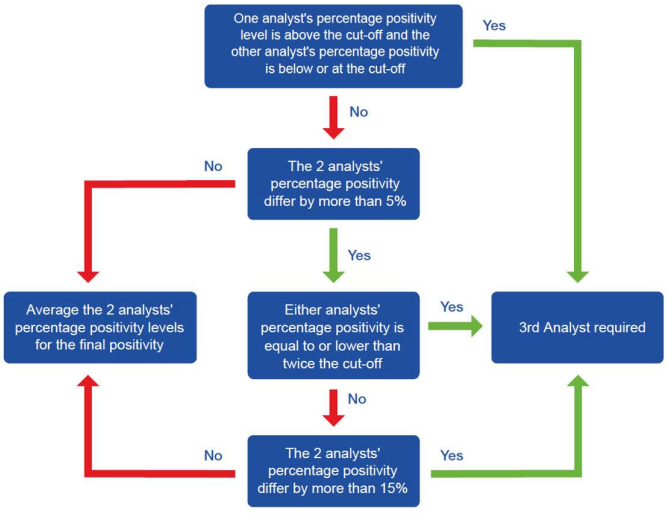
Third Analyst Requirements

Depending on the number of abnormal nuclei each analyst has seen, a third analyst may be required. The third analyst will analyse the same number of nuclei as the first two analysts as specified by institutional, regional or national guidelines. The rules for a third analyst are detailed in the text and flowchart below.

A third analyst is required if:

- One of the analysts' percentage positivity is above the cut-off and the other analysts' percentage positivity is below or at the cut-off.
- The two analysts' percentage positivity differ by more than 5% and either analyst's percentage positivity is equal to or lower than twice the cut-off.
- The two analysts' percentage positivity differ by more than 15%.

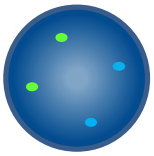
Of the three scores obtained, the two closest positive percent values will be averaged for the final positivity. If the 3 scores are equidistant then the median positivity value should be used.



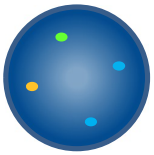
Expected Results

LPA002 Prenatal X, Y and 18 Enumeration Probe

Expected Normal Signal Patterns

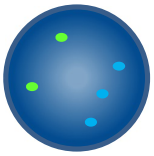


In a normal female cell, two green and two aqua signals are expected (2G2A).

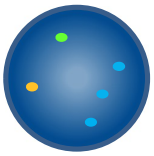


In a normal male cell, one green, one orange and two aqua signals are expected (1G1O2A).

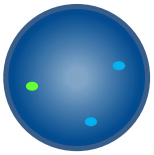
Expected Abnormal Signal Patterns



In a female cell with trisomy 18, the expected signal pattern will be two green signals and three aqua signals (2G3A).



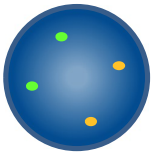
In a male cell with trisomy 18, the expected signal pattern will be one green signal, one orange signal and three aqua signals (1G1O3A)



In a cell with Turners syndrome, the expected signal pattern will be one green signal and two aqua signals (1G2A).

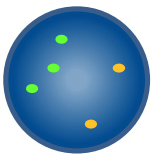
LPA003 Prenatal 13 and 21 Enumeration Probe

Expected Normal Signal Pattern

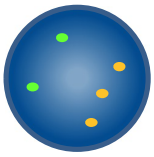


In a normal cell, two green and two orange signals are expected (2G2O).

Expected Abnormal Signal Patterns



In a cell with trisomy 13, three green signals and two orange signals will be expected (3G2O).



In a cell with trisomy 21, two green signals and three orange signals will be expected (2G3O).

Other signal patterns are possible in aneuploid/unbalanced specimens.

Known Relevant Interferences / Interfering Substances
No known relevant interferences / interfering substances.

Known Cross-Reactivity
The DXZ1 probe may show cross-hybridisation to the centromeres of chromosomes 1, 11, 17 and Y.
The DYZ3 probe may show cross-hybridisation to the centromeres of chromosomes 13, 14, 15, 20, 21, 22 and X.
The D18Z1 probe may show cross-hybridisation to the centromeres of chromosomes 2, 4, 8, 9, 13, 14, 15, 20, 21 and 22.

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@oqt.com
For EU National Competent Authorities, a list of vigilance contact points can be found at:
https://health.ec.europa.eu/medical-devices-sector/new-regulations/contacts_en

Specific Performance Characteristics
Analytical Specificity
LPA002 Prenatal X, Y and 18 Enumeration Probe
Analytical specificity is defined as the percentage of signals that hybridise to the correct locus and no other location. 4 chromosomal loci in each of 20 metaphase cells from 5 samples were analysed, giving 100 data points each for chromosome X and Y and 200 data points for chromosome 18. The location of each hybridised probe was mapped and the number of metaphase chromosome FISH signals that hybridised to the correct locus was recorded.

The analytical specificity of each probe in the kit was calculated as the number of metaphase chromosome FISH signals hybridised to the correct locus divided by the total number of metaphase chromosome FISH signals hybridised. This result was expressed as a percentage with a 95% confidence interval.

Table 1. Analytical Specificity for the LPA002 Prenatal X, Y, and 18 Enumeration Probe

Target	Number of metaphase chromosomes hybridised	Number of correctly hybridised loci	Analytical Specificity	95% Confidence Interval
Xp11.1-q11.1	100	100	100%	96.30 - 100%
Yp11.1-q11.1	100	100	100%	96.30 - 100%
18p11.1-q11.1	200	200	100%	98.12 - 100%

LPA003 Prenatal 13 and 21 Enumeration Probe
Analytical specificity is defined as the percentage of signals that hybridise to the correct locus and no other location. 4 chromosomal loci in each of 20 metaphase cells from 5 samples were analysed, giving 200 data points each for chromosomes 13 and 21. The location of each hybridised probe was mapped and the number of metaphase chromosome FISH signals that hybridised to the correct locus was recorded.

The analytical specificity of each probe in the kit was calculated as the number of metaphase chromosome FISH signals hybridised to the correct locus divided by the total number of metaphase chromosome FISH signals hybridised. This result was expressed as a percentage with a 95% confidence interval.

Table 2. Analytical Specificity for the LPA003 Prenatal 13 and 21 Enumeration Probe

Target	Number of metaphase chromosomes hybridised	Number of correctly hybridised loci	Analytical Specificity	95% Confidence Interval
13q14.2	200	200	100%	98.12 - 100%
21q22.1	200	200	100%	98.12 - 100%

Analytical Sensitivity
LPA002 Prenatal X, Y and 18 Enumeration Probe
Analytical sensitivity is the percentage of scoreable interphase cells with the expected normal signal pattern. A minimum of 50 interphase cells were analysed for each of 25 fixed cell suspensions from amniotic fluid samples from karyotypically normal males and 25 karyotypically normal females deemed as having a normal complement of chromosome 18, resulting in a minimum of 2500 nuclei scored overall. The sensitivity data was analysed based on the percentage of cells showing a normal expected signal pattern and expressed as a percentage with a 95% confidence interval.

Table 3. Analytical Sensitivity for the LPA002 Prenatal X, Y, and 18 Enumeration Probe

Sample Type	Sensitivity Criteria	Sensitivity Result
Amniotic fluid	>95%	95.99% (95.15-96.69%)

LPA003 Prenatal 13 and 21 Enumeration Probe
Analytical sensitivity is the percentage of scoreable interphase cells with the expected normal signal pattern. A minimum of 50 interphase cells were analysed for each of 25 fixed cell suspensions from amniotic fluid samples from karyotypically normal males or females deemed as having a normal complement of chromosomes 13 and 21, resulting in a minimum of 1250 nuclei scored for each sample type. The sensitivity data was analysed based on the percentage of cells showing a normal expected signal pattern and expressed as a percentage with a 95% confidence interval.

Table 4. Analytical Sensitivity for the LPA003 Prenatal 13 and 21 Enumeration Probe

Sample Type	Sensitivity Criteria	Sensitivity Result
Amniotic fluid	>95%	96.24% (94.84-97.64%)

Characterisation of Normal Cut-off Values
LPA002 Prenatal X, Y and 18 Enumeration Probe
The normal cut-off is defined as the percentage of cells exhibiting a false positive signal pattern at which an individual would be considered normal and not consistent with a clinical diagnosis. A minimum of 50 interphase cells were analysed for each of 25 fixed cell suspensions from amniotic fluid samples from karyotypically normal males and 25 karyotypically normal females deemed as having a normal complement of chromosome 18, resulting in a minimum of 2500 nuclei scored overall.

The cut-off value was determined using the β-inverse (BETAINV) function in MS Excel. It was calculated as the percentage of interphase cells showing a false positive signal pattern using the upper bound of a one-sided 95% confidence interval of the binomial distribution in a normal patient sample.

Table 5. Characterisation of Normal Cut-off Values for the LPA002 Prenatal X, Y, and 18 Enumeration Probe

Sample Type	Signal pattern	Cut-off Result
Amniotic fluid	1G1O3A	11.62%
	2G3A	8.97%
	1G2A	13.98%

LPA003 Prenatal 13 and 21 Enumeration Probe
The normal cut-off is defined as the percentage of cells exhibiting a false positive signal pattern at which an individual would be considered normal and not consistent with a clinical diagnosis. A minimum of 50 interphase cells were analysed for each of amniotic fluid cell suspensions from 25 karyotypically normal males or females, resulting in a minimum of 1250 nuclei scored for each sample type.

The cut-off value was determined using the β-inverse (BETAINV) function in MS Excel. It was calculated as the percentage of interphase cells showing a false positive signal pattern using the upper bound of a one-sided 95% confidence interval of the binomial distribution in a normal patient sample.

Table 6. Characterisation of Normal Cut-off Values for the LPA003 Prenatal 13 and 21 Enumeration Probe

Sample Type	Signal pattern	Cut-off Result
Amniotic fluid	2G3O	8.97%
	3G2O	

Laboratories must verify cut-off values using their own data.[13,14]

Precision
LPA002 Prenatal X, Y and 18 Enumeration Probe
The precision of this product has been measured in terms of intra-day precision (sample-to-sample), inter-day precision (day-to-day) and single-site inter-lot precision (lot-to-lot).

3 samples were used to assess the precision of this product: 1 normal male amniotic fluid sample deemed as XY and having a normal complement of chromosome 18, 1 normal female amniotic fluid sample deemed as XX and having

a normal complement of chromosome 18, and 1 low positive female trisomy 18 amniotic fluid sample.

Inter-day and intra-day precision were evaluated by testing samples in triplicate across 10 non-consecutive days. Lot-to-lot precision was evaluated by testing 3 independent product lots in triplicate using the same samples. Results were expressed as overall agreement with the expected classification (positive or negative).

Table 7. Reproducibility and Precision for the LPA002 Prenatal X, Y, and 18 Enumeration Probe

Variable	Sample type	Agreement
Intra-day	Female negative amniotic fluid	100.0%
	Female trisomy 18 amniotic fluid	90.0%
Inter-day	Female negative amniotic fluid	100.0%
	Female trisomy 18 amniotic fluid	90.0%
	Male negative amniotic fluid	100.0%
Inter-lot	Female negative amniotic fluid	100.0%
	Female trisomy 18 amniotic fluid	100.0%
	Male negative amniotic fluid	100.0%

LPA003 Prenatal 13 and 21 Enumeration Probe
The precision of this product has been measured in terms of intra-day precision (sample-to-sample), inter-day precision (day-to-day) and single-site inter-lot precision (lot-to-lot).

3 samples were used to assess the precision of this product: 1 normal amniotic fluid sample deemed as having a normal complement of chromosomes 13 and 21, one 1 low positive trisomy 13 amniotic fluid sample, and 1 low positive trisomy 21 amniotic fluid sample.

Inter-day and intra-day precision were evaluated by testing samples in triplicate across 10 non-consecutive days. Lot-to-lot precision was evaluated by testing 3 independent product lots in triplicate using the same samples. Results were expressed as overall agreement with the expected classification (positive or negative).

Table 8. Reproducibility and Precision for the LPA003 Prenatal 13 and 21 Enumeration Probe

Variable	Sample type	Agreement
Intra-day and inter-day	Negative amniotic fluid	100.0%
	Low positive trisomy 13 amniotic fluid	100.0%
	Low positive trisomy 21 amniotic fluid	96.7%
Inter-lot	Negative amniotic fluid	88.9%
	Low positive trisomy 13 amniotic fluid	100.0%
	Low positive trisomy 21 amniotic fluid	100.0%

Clinical Performance
LPA002 Prenatal X, Y and 18 Enumeration Probe
To ensure that the Prenatal X, Y and 18 Enumeration Probe detects intended rearrangements, clinical performance was established over one study on representative samples of the intended population for the product: Residual 3:1 methanol/acetic acid-fixed material from prenatal amniotic fluid samples. The sample size for the study was 71 specimens, with a population of 3 trisomy 18 positive specimens, 2 monosomy X positive specimens, and 66 negative specimens. All samples were de-identified and randomised to prevent analysis bias. The results were compared to the known status of the sample. The probe correctly identified the status of the samples in all instances.

The results of these tests were analysed in order to provide clinical sensitivity, clinical specificity and false positive rate (FPR) values for positive signals, using a one-dimensional approach.

Table 9. Clinical Performance for the Prenatal X, Y and 18 Enumeration Probe

Variable	Result
Clinical Sensitivity (true positive rate, TPR)	100.0%
Clinical Specificity (true negative rate, TNR)	100.0%
False Positive Rate (FPR) = 1 – Specificity	0.0%

LPA003 Prenatal 13 and 21 Enumeration Probe
To ensure that the Prenatal 13 and 21 Enumeration Probe detects intended rearrangements, clinical performance was established over three studies on representative samples of the intended population for the product: Residual 3:1 methanol/acetic acid-fixed material from prenatal amniotic fluid samples. The sample size for the study was 172 specimens, with a population of 15 trisomy 13 positive and 157 trisomy 13 negative specimens, and a total of 109 trisomy 21 positive and 63 trisomy 21 negative specimens. The results were compared to the known status of the sample. The probe correctly identified the status of the samples in all instances.

The results of these tests were analysed in order to provide clinical sensitivity, clinical specificity and false positive rate (FPR) values for positive signals, using a one-dimensional approach.

Table 10. Clinical Performance for the Prenatal 13 and 21 Enumeration Probe

Variable	Result
Clinical Sensitivity (true positive rate, TPR)	100.0%
Clinical Specificity (true negative rate, TNR)	100.0%
False Positive Rate (FPR) = 1 – Specificity	0.0%

Summary of Safety and Performance (SSP)
The SSP shall be made available to the public via the European database on medical devices (Eudamed), where it is linked to the Basic UDI-DI.
Eudamed URL: https://ec.europa.eu/tools/eudamed
Basic UDI-DI: 50558449LPA001GG

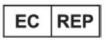
If Eudamed is not fully functional, the SSP shall be made available to the public upon request by emailing SSP@ogt.com.

Additional Information
For additional product information, please contact the CytoCell Technical Support Department.
T: +44 (0)1223 294048
E: techsupport@cytocell.com
W: www.ogt.com

References

1. Alliance G,(2010). Chromosomal Abnormalities [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK132134/.
2. Dey M, et al., (2013). Prenatal screening methods for aneuploidies. North American Journal of Medical Sciences .
3. Antonarakis SE, Strydom A., (2020). Down Syndrome. Nature Reviews Disease Primers. .
4. Orphanet: Down syndrome [Internet]. www.orpha.net. Available from: https://www.orpha.net/en/disease/detail/870.
5. Orphanet: Trisomy 13 [Internet]. www.orpha.net. Available from: https://www.orpha.net/en/disease/detail/3378.
6. Pyle AK, et al. (2025) Guidance for Caring for Infants and Children With Trisomy 13 and Trisomy 18: Clinical Report. PEDIATRICS. .
7. Balasundaram P, et al. (2023). Edwards syndrome. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–. Updated 2025 Feb 15. Available from: https://www.ncbi.nlm.nih.gov/books/NBK570597/.
8. Shankar Kikkeri N, et al,. (2023). Turner Syndrome; Available from: https://www.ncbi.nlm.nih.gov/books/NBK554621/.
9. Best Practice Guidelines (2024). The Association for Clinical Genomic Science (ACGS). Cytogenetics Constitutional Guidelines.
10. NHS England - National genomic test directory for rare and inherited disease. Available from: https://www.england.nhs.uk/publication/national-genomic-test-directories/
11. Silva M. et al. (2019) European guidelines for constitutional cytogenomic analysis. European Journal of Human Genetics.
12. Arsham, MS., Barch, MJ. and Lawce HJ. (eds.) (2017) *The AGT Cytogenetics Laboratory Manual*. New Jersey: John Wiley & Sons Inc. References
13. Mascarello JT, Hirsch B, Kearney HM, et al. Section E9 of the American College of Medical Genetics technical standards and guidelines: fluorescence in situ hybridization. Genet Med. 2011;13(7):667-675.
14. Wiktor AE, Dyke DLV, Stupca PJ, Ketterling RP, Thorland EC, Shearer BM, Fink SR, Stockero KJ, Majorowicz JR, Dewald GW. *Preclinical validation of fluorescence in situ hybridization assays for clinical practice*. Genetics in Medicine. 2006;8(1):16–23.

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
	**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
	en: Caution	5.4.4
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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