EmbryoCellect™
Pre-implantation Genetic Screening Kit

TECHNICAL INFORMATION
Aneuploidy

Whole chromosome aneuploidy has been shown to affect all chromosomes in IVF embryos. Aneuploidy is a significant cause of IVF failure, especially in women of advanced maternal age.

Pre-implantation genetic screening (PGS)

Initial attempts to detect aneuploidy in IVF embryos used FISH screening for a limited subset of chromosomes (5-12 chromosomes only). Clinical data from these first attempts showed no benefit to IVF success rates.

This has changed dramatically since the introduction of advanced 24 chromosome pre-implantation genetic screening (PGS). PGS now assesses the loss or gain of any whole chromosomes.

PGS can increase the clinical pregnancy rate by around 50%


Selecting only euploid embryos to transfer has been demonstrated to:

- reduce the time to pregnancy;
- reduce the incidence of miscarriage;
- achieve comparable single embryo transfer clinical pregnancy rates to unscreened multiple embryo transfer;
- allow the selection of euploid embryos for vitrification (freezing) avoiding the expense of storing embryos not suitable for transfer; and
- overcome the maternal age impact on IVF success.

96% of aneuploid embryos fail to implant

Whole Genome Amplification (WGA)

Typically only a few or even a single cell are used for pre-implantation genetic screening (PGS). A single human cell contains approximately 6 picograms ($6 \times 10^{-12}$ grams) of DNA that needs to be reliably amplified (copied) millions of times to obtain enough DNA for screening. This process, which is termed whole genome amplification (WGA), is performed using a specialised type of polymerase chain reaction (PCR). The robustness and fidelity of the WGA is very important, as any errors introduced by this process may affect the accuracy of the results. RHS have used advanced PCR polymerases to optimise the WGA in EmbryoCellect™.

Array Comparative Genomic Hybridisation (aCGH)

aCGH is used to determine the number of chromosomes in a sample by comparing the relative amount of fluorescence of the sample to a known reference.

The aCGH process

A sample (embryo biopsy) is placed into a PCR tube and enzymatically lysed. WGA amplifies the genome millions of times.

The reference sample is labelled with a red fluorescent dye.

The test sample is labelled with a green fluorescent dye.

The test and reference sample are combined on the microarray surface and they compete for binding positions.

After microarray scanning and data analysis, the relative fluorescence signals of the test and reference are compared.

Outliers: extra copies of chromosomes 16 & 21 (green signal greater than red signal)

Equal number of chromosomes (green signal equal to red signal)

This EmbryoCellect™ result was generated from a single fibroblast from a male cell line with trisomy in chromosomes 16 and 21 (48,XY,+16,+21)
What is printed on the **EmbryoCellect™** microarray?

Multiple copies of single metaphase chromosomes are laser captured. Whole chromosome probes containing on average 1.2 million unique sequences per chromosome are printed onto microarray slides. There are 4 microarrays (test areas) per slide, each microarray containing probes for all 24 human chromosomes in replicates of 8.

Individual chromosomes are whole genome amplified using the RHS DOP-PCR and then repeat depleted and printed.

The **EmbryoCellect™** microarray is fundamentally different to other microarrays.

Unlike BAC or oligonucleotide arrays, where each probe contains a single DNA target ranging in size from approximately 60-150 basepairs, each feature (spot) on the EmbryoCellect™ array contains a whole chromosome library. This provides on average 1.2 million unique chromosome-specific target fragments of DNA ranging in size from approximately 200 to 4,000 basepairs.

This approach (single cell microarray to detect aneuploidy in embryos) was first described by RHS scientists from the Department of Obstetrics and Gynaecology, The University of Adelaide in 2004 and 2007 and is exclusively licensed to RHS.

This patented approach allows the EmbryoCellect™ microarray to collect test and reference signal from an entire chromosome in a single result providing a clear indication of whole chromosome count.

Product attributes

**What is printed on the array?**
Chromosome-specific DOP-amplified and repeat deleted PCR products ranging in size from 120bp – 4kb

**How are they printed?**
Spot printed as a pooled library of sequences specific to each chromosome

**Number of array targets**
Over 35 million sequences per array with, on average, 1.2 million chromosome specific sequences per spot.

**Replicate targets on chip**
One spot for each of the 24 human chromosomes; Eight replicates per array

**Microarrays per slide**
Four allowing the testing of as few as four samples at a time. One operator can manage 4 slides in a batch if required

**Ease of analysis**
There is a single spot per chromosome so there is no need to calculate a consensus across the chromosome to detect aneuploidy. The results are very clear and specific for the detection of whole chromosome aneuploidy.

**EmbryoCellect™** has been specifically developed to screen for whole chromosome aneuploidy in single or small numbers of cells.
Why EmbryoCellect™?

The test is simple and robust
The results are easy to interpret
The test has been validated for accuracy
The test has been designed for labs to bring testing in-house by providing the flexibility to run small numbers of samples
The raw scanner data is available for IVF labs to use for internal QC, if required

The EmbryoCellect™ kit contents

- Cell lysis buffer and enzyme for lysing the sample cells
- Reference male gDNA
- Whole Genome Amplification (WGA) reagents
- Fluorescent labelling PCR reagents
- Five patented EmbryoCellect™ microarray slides with four microarrays per slide
- Software: A Simple Excel macro that uses a microarray scanner-generated data file

The EmbryoCellect™ kit contains enough reagents to test 20 samples
<table>
<thead>
<tr>
<th>Protocol Step</th>
<th>Explanation</th>
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<tr>
<td>Cell lysis 15 mins</td>
<td>Following biopsy, a gentle but effective enzyme-based lysis procedure ensures robust cell lysis and a readily accessible DNA template for whole genome amplification.</td>
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<td>Whole genome amplification 2.5 hrs</td>
<td>Whole genome amplification is performed using RHS’s DOP-PCR, which has been optimised for the RHS microarray. DOP-PCR uses degenerate primers to initiate DNA amplification, binding across a broad range of different sequences scattered genome wide.</td>
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<td>Agarose gel assessment 30 mins</td>
<td>Following amplification, the use of agarose gel electrophoresis is recommended to ensure that cell amplification has been successful. (30 mins)</td>
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<td>Labelling PCR 45 mins</td>
<td>Successfully amplified samples are fluorescently labelled by a second DOP-PCR. The test is labelled with a Cy3 equivalent dye and the reference with a Cy5 equivalent dye.</td>
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<td>Clean-up and nanodrop 30 mins</td>
<td>Once purified, these labelled amplicons are again assessed using agarose gel electrophoresis and spectrophotometry to ensure adequate amplification and dye incorporation has occurred.</td>
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<td>Hybridisation 3 hrs to overnight</td>
<td>Samples are competitively hybridized to the RHS microarray.</td>
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<td>Microarray washing 30 mins</td>
<td>After incubation, the microarray is washed and scanned. The ratio of test to reference dye intensity after normalization is determined using RHS proprietary software, providing the ploidy status of each chromosome in each test sample.</td>
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Reproductive Health Science Ltd is a developer of advanced single cell genomic technologies with a focus on improving health and research outcomes.

Further background reading


Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Fertility and Sterility 2013 Sep;100(3):624-30

Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, Tao X, Treff NR. Fertility and Sterility 2013 Sep;100(3):697-703

In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, Treff NR, Scott RT Jr. Fertility and Sterility July 2013 100-7


RHS inventor publications

