



**EuroClone**<sup>®</sup>  
EUROCLONE S.p.A.

# CHROMOSOME KIT P & M

Ready to use media for Cytogenetic analysis of peripheral blood and bone marrow cells



# SYNCHROSET

Reagents for cell Synchronization



**1. Chromosome kit P & M/Chromosome Media P & M and Synchroset**

## INTRODUCTION

### Cytogenetic analysis

The aim of Cytogenetic investigation is to study the cellular chromosome complement as an organised representation of the nuclear genome. The chromosome content or "karyotype" is classified on the basis of both chromosome number and morphology, which are fixed characteristics for a particular species. In the human species for example, every cell (except germ cells) contains 46 chromosomes grouped as 23 pairs.

As a result of mutations, the karyotype may undergo numerical as well as morphological changes giving rise to "chromosome numerical aberrations" and "chromosome structural aberrations", respectively.

### Diagnostic cytogenetics

The identification and characterisation of constitutional abnormalities (abnormalities present in the zygote or appearing in a subsequent ontogenetic step) and acquired chromosome abnormalities (abnormalities restricted either to a particular tissue or to a cell lineage) are the focus of two large areas of diagnostic investigation (the latter however being essentially represented by Cancer cytogenetics). Another field of study, Cytogenetic mutagenesis, is closely related to both areas. Although not necessarily the subject of diagnostic investigations, it deals mainly with the recognition and analysis of chromosome aberrations induced by environmental agents such as ionizing radiation as well as chemical and viral agents.

### Cell types suitable for chromosome analysis in post-natal diagnostics

Chromosome preparations may be obtained via the culture of a variety of tissues; in humans, the following sources are used for the majority of diagnostic purposes:

- short-term cultures of whole blood
- long-term cultures of skin fibroblasts
- direct preparations of bone marrow

## CHROMOSOME KIT and Medium P: GENERAL DESCRIPTION

The **Chromosome Kit P** was developed to optimize the preparation of cultures of peripheral blood lymphocytes for subsequent chromosome analysis.

Special attention has been paid to the sample / medium volume with respect to the size of the culture surface area which together with the particular composition of the culture medium leads to an optimal mitotic index.

The **Chromosome Kit P** is a sterile complete medium (there is no requirement to add other components) which is supplied ready to use in a TC test tube; the same medium is supplied also in a bottle of 100 ml or 500 ml.

Its special formulation also makes it suitable for cultures of foetal blood lymphocytes obtained via cordocentesis.

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The **Chromosome Kit P** contains:

- RPMI 1640
- 10% Foetal bovine serum
- L-Glutamine
- Gentamicin
- HEPES
- Heparin
- Interleukine
- PHA P-form

### **CHROMOSOME KIT and Medium M: GENERAL DESCRIPTION**

The **Chromosome Kit M** was developed to optimize the preparation of bone marrow cell cultures for subsequent chromosome analysis.

Special attention has been paid to the sample / medium volume with respect to the size of the culture surface area which together with the particular composition of the culture medium leads to an optimal mitotic index.

The **Chromosome Kit M** is a sterile complete medium (there is no requirement to add other components) which is supplied ready to use in a TC test tube; the same medium is supplied also in a bottle of 100 ml or 500 ml.

The **Chromosome Kit M** contains:

- RPMI 1640
- 20% foetal bovine serum
- L-Glutamine
- Gentamicin
- HEPES
- Heparin
- Growth Factor

The use of the **Chromosome Kit M** test tube allows a substantial reduction in processing and handling procedures, which directly benefits laboratory routines. The use of **Chromosome Kit M** results in simplified working protocols, and guarantees that microscopic observations are performed on reproducible and high quality samples: the number of metaphases and chromosome quality are in fact significantly higher when compared to those obtained by the use of either conventional media or with other commercial kits.

#### **Product stability**

The **Chromosome Kit M** is available in packages of 20 or 50 tubes and is shipped at +4°C conditions, it may be stored for a maximum of 30 days. However, in order to maintain the trophic features of the medium until the expiry date printed on the label, freezing at -20°C is highly recommended.

The evaluation tests performed to date have shown that this product is stable, if correctly stored, for at least 6 months following the date of production.

## **CYTOGENETIC ANALYSIS OF BONEMARROW BLOOD SAMPLES**

Bone marrow is used for chromosome analysis in the case of pathological conditions associated with abnormal proliferation of the haematopoietic tissue. Due to the presence of spontaneous mitosis, it is unnecessary to set up an *in vitro* cell culture and only a short incubation is therefore required. This has the advantage of not being selective for cell lines bearing an abnormal chromosome set.

Before starting the culture, it is advisable to attempt an assessment of the number of nucleated cells contained in the bone marrow blood sample (the optimal concentration of nucleated cells is  $1 \times 10^6$  cells per ml of culture).

### **Cell counting**

- Dilute 0.1 ml of sample to 2 ml with Turck's solution .
- Take one drop of the diluted suspension and place it in a Burker chamber.
- Count the cells observed in 4 of the 3-line delimited squares.
- Divide this number by 4 and multiply by  $2 \times 10^5$  (= number of cells per ml).

## **CELL SYNCHRONIZATION USING SYNCHROSET**

In order to obtain chromosome preparations with a high number of metaphases and outstanding quality, the use of **Synchroset**, in combination with either **Chromosome Kit P** or **Chromosome Kit M** is recommended.

Using the two solutions, "A" and "B", which are provided as part of the kit, and an extremely simple protocol, synchronization of the cell cycle in both lymphocytes and bone marrow cells becomes straightforward. The **Synchroset** may also be used for the production of high numbers of prometaphases in which the chromosomes are suitable for high resolution banding techniques. In these preparations the individual bands normally visualized with standard methods may be divided into sub-bands (up to 850-1.000 per haploid set).

This methodology is suitable for more precise identification of breakpoints and minor abnormalities such as micro deletions, which often cause severe pluriformative conditions.

### **Product stability**

**Synchroset** is composed of 4 vials containing 1.5 ml of Solution "A" and 4 vials containing 1.5 ml of Solution "B".

The solutions "A" and "B" should be kept at  $-20^{\circ}\text{C}$  and protected from light.

Repeated freezing/thawing cycles will not affect performance.

Tests have shown that this product, if appropriately stored, is stable for at least six months following the date of production.

### **Use of Synchroset with lymphocyte cultures**

- 1) Before leaving the lab in the evening and after 48-56 hours of culture, add 0.1 ml of solution "A" to each culture and incubate overnight.
- 2) The following morning, add 0.1 ml of solution "B" to each culture and incubate for a further 5 hours (it is not necessary to wash the cells).
- 3) Add one or two drops of Colcemid and keep the tubes in the incubator for another 60 minutes (approx.) This last incubation time may be reduced to 15 minutes if the aim is to obtain a higher number of prometaphases.
- 4) The chromosome preparation may be performed according to the protocol.

### **Use of Synchroset in bone marrow cultures**

- 1) Before leaving the lab in the evening add 0.1 ml of solution "A" to each culture and incubate overnight.
- 2) The following morning, add 0.1 ml of solution "B" to the cultures and incubate for a further 6-8 hours (it is not necessary to wash the cells).
- 3) Add one or two drops of Colcemid during the last hour of incubation.
- 4) Prepare the chromosomes according to the protocol.

*Note: After cell synchronisation, chromosome preparations may require longer incubation times with trypsin to obtain a satisfactory GTG or RHG banding pattern.*

### **Synchroset contains:**

- Thymidine
- Fluorodeoxyuridine

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## Euroclone products for Cytogenetics

| Cat. N°     | Description   | Pack    |
|-------------|---|---------|
| EK AMT-500  | <b>Chromosome Kit "P"</b>   | 50 test |
| EK AMT-200  | Sterile ready-to-use test tubes, with 5 ml<br>Complete medium for human lymphocyte culture  | 20 test |
| EK AMT-500M | <b>Chromosome Kit "M"</b>   | 50 test |
| EK AMT-200M | Sterile ready-to-use test tubes, with 5 ml<br>Complete medium for bone marrow cell culture  | 20 test |
| EK AMTS-008 | <b>Synchroset</b><br>Reagents for synchronisation of lymphocytes<br>And bone marrow cell populations  | 50 test |
| EK AMN-240  | <b>Amniodish</b><br>35 mm petri dishes with slide, gamma sterile,<br>for culture of cells from amniotic fluid, chorionic villi,<br>solid tumors and other tissues | 40x6 pz |
| EK AMG-100  | <b>Amniomed</b>   | 100 ml  |
| EK AMG-500  | Complete medium for amniotic fluid, chorionic villi<br>and foetal tissues. Sterile and ready-to-use   | 500 ml  |
| EK AMH-950  | <b>Optichrome</b><br>An instrument designed to optimize the quality of<br>chromosome analysis in a controlled environment   | 1 pz    |
| EK AMH-900  | <b>Quickchrome</b><br>A multislides-making instrument designed for direct<br>chromosome preparation from chorionic villi and solid tumor                          | 1 pz    |