

## TERGUM S.L. AGREEMENT

**TITLE:** BENEFICIAL PROPERTIES OF TERGUM MAXIMUM (TM) AGAINST OXIDATIVE STRESS, CELL DEATH AND CELL PROLIFERATION IN FIBROBLAST AND KERATINOCYTES

**SCIENTIFIC PARTNER IN THE IBiS:** Laboratory 209

**FUNDING COMPANY:** TERGUM S.L. C.I.F. B91875658 que se encuentra domiciliada en Av. Veintiocho de Febrero 39, 41702 Dos Hermanas.

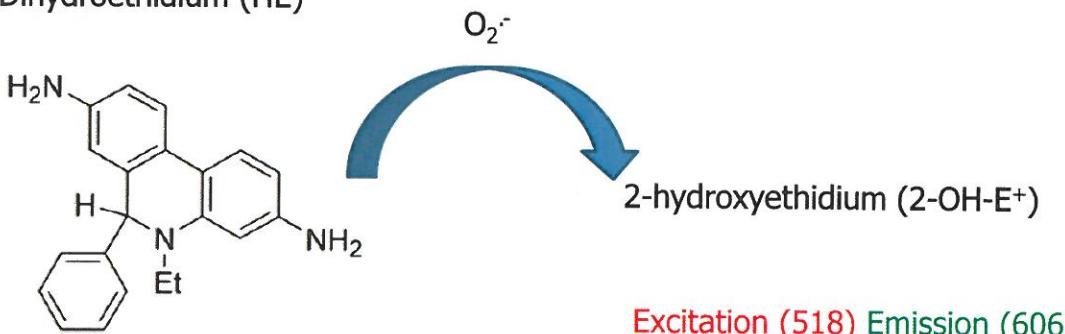
## SCIENTIFIC REPORT

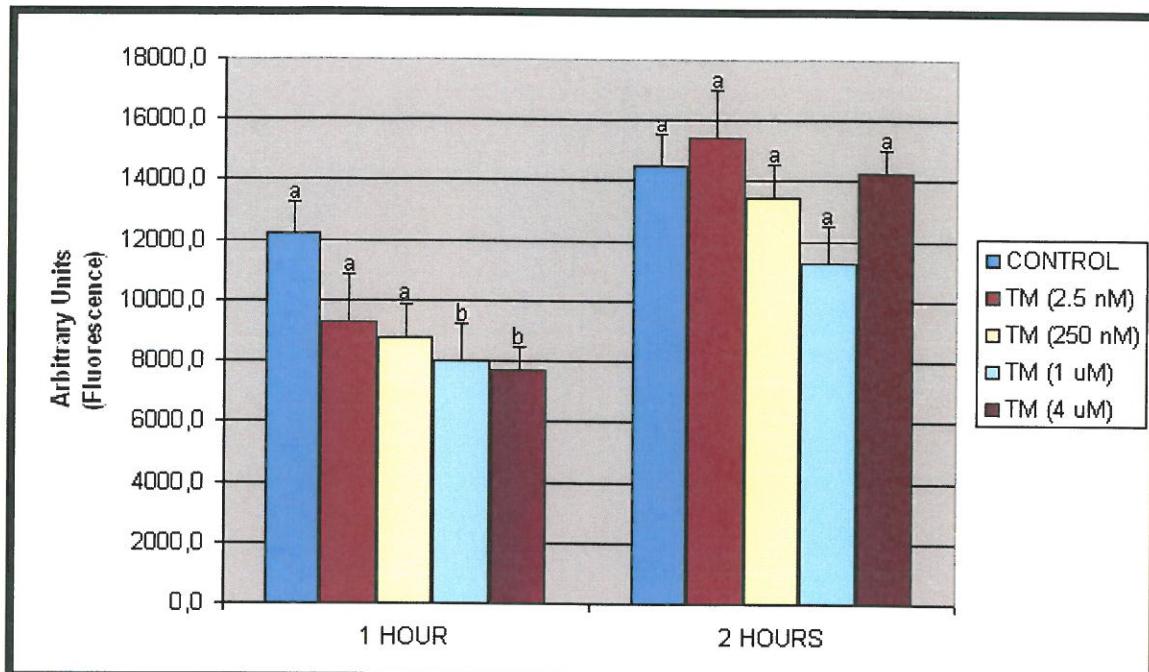
### 1) OXIDATIVE AND NITROSATIVE STRESS

It has been measured the effect of the Tergum Maximum (TM) on the intracellular superoxide anion production in fibroblasts.

Dihydroethidium (Dhe, D11347, Life) is a highly fluorescent molecule which is useful for the measurement of superoxide anion production following the reaction:

Dihydroethidium (HE)

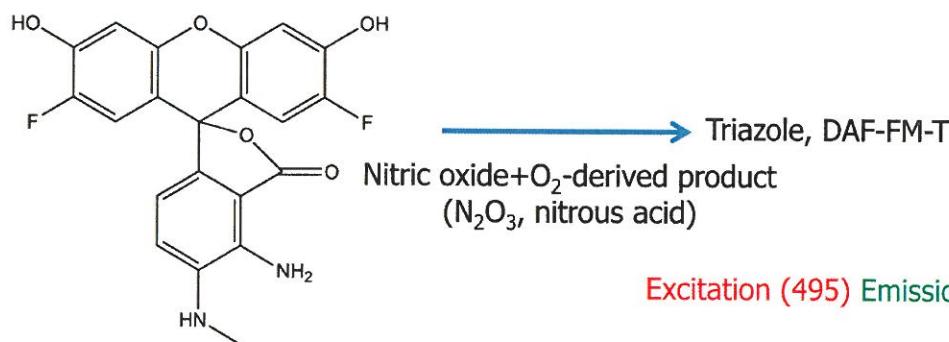


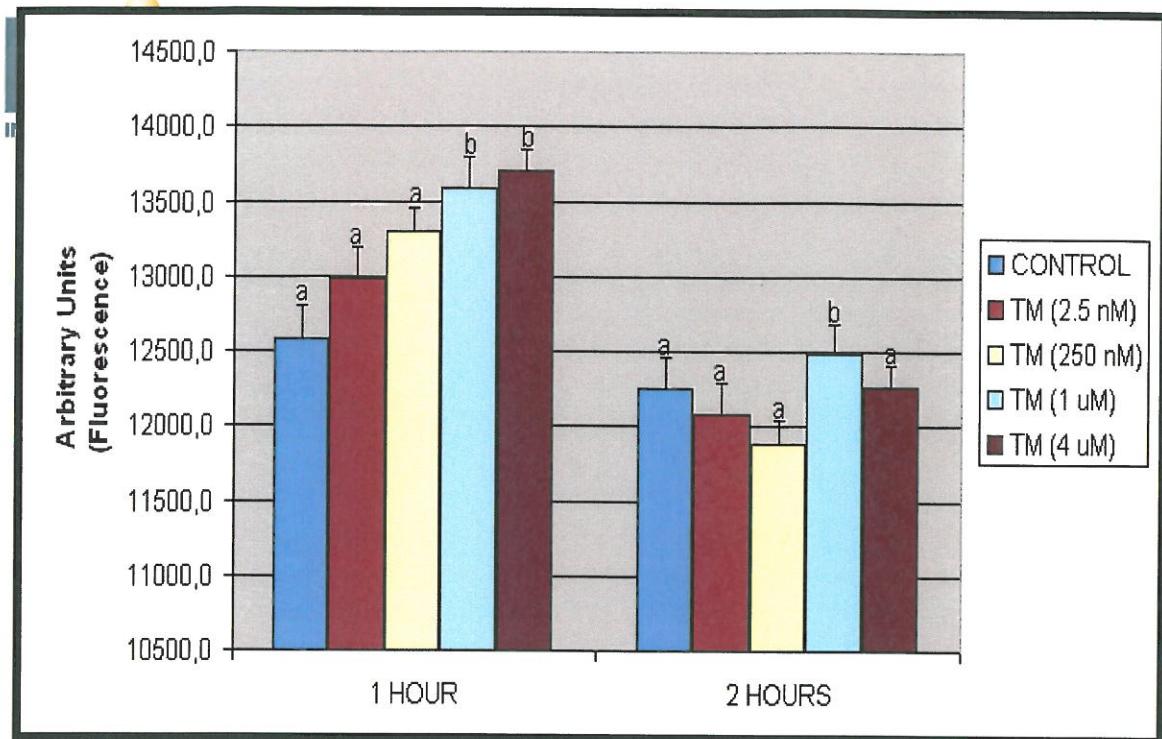


**Figure 1:** Regulation of superoxide anion production by TM in fibroblast

4-amino-5-methylamino-20,70-difluorofluorescein (DAF-FM, D23842, Life) is a highly fluorescent molecule which is used to measure nitric oxide production following the reaction.

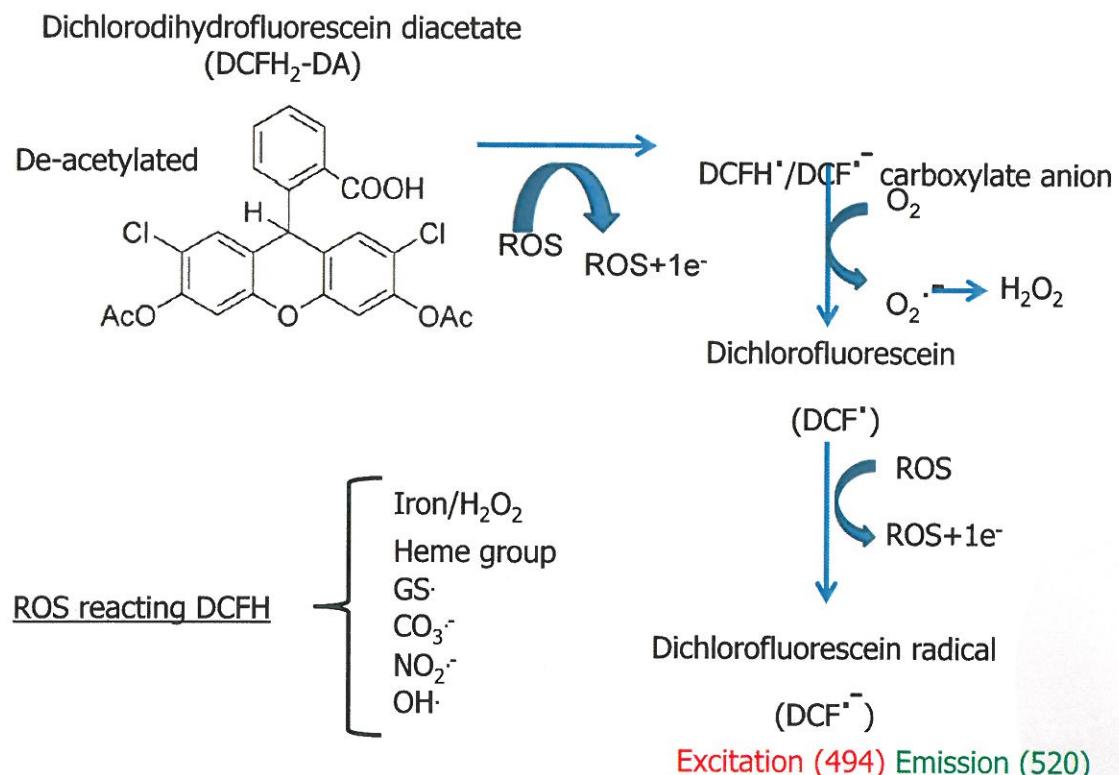
**4-amino-5-methylamino-20,70-difluorofluorescein**

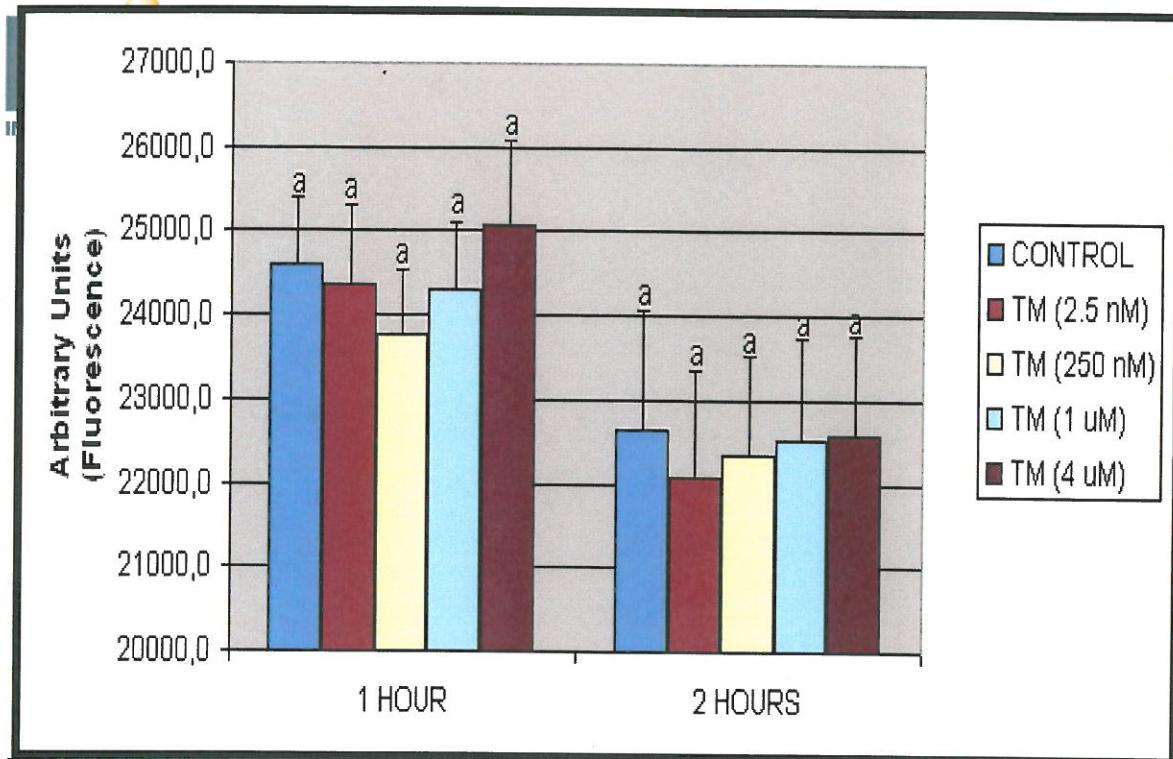




**Figure 2:** Regulation of nitric oxide production by TM in fibroblast

Dichlorodihydrofluorescein diacetate (DCFH<sub>2</sub>-DA, D399, Life) is a highly fluorescent molecule which is used to measure reactive oxygen species (ROS) following the reaction:

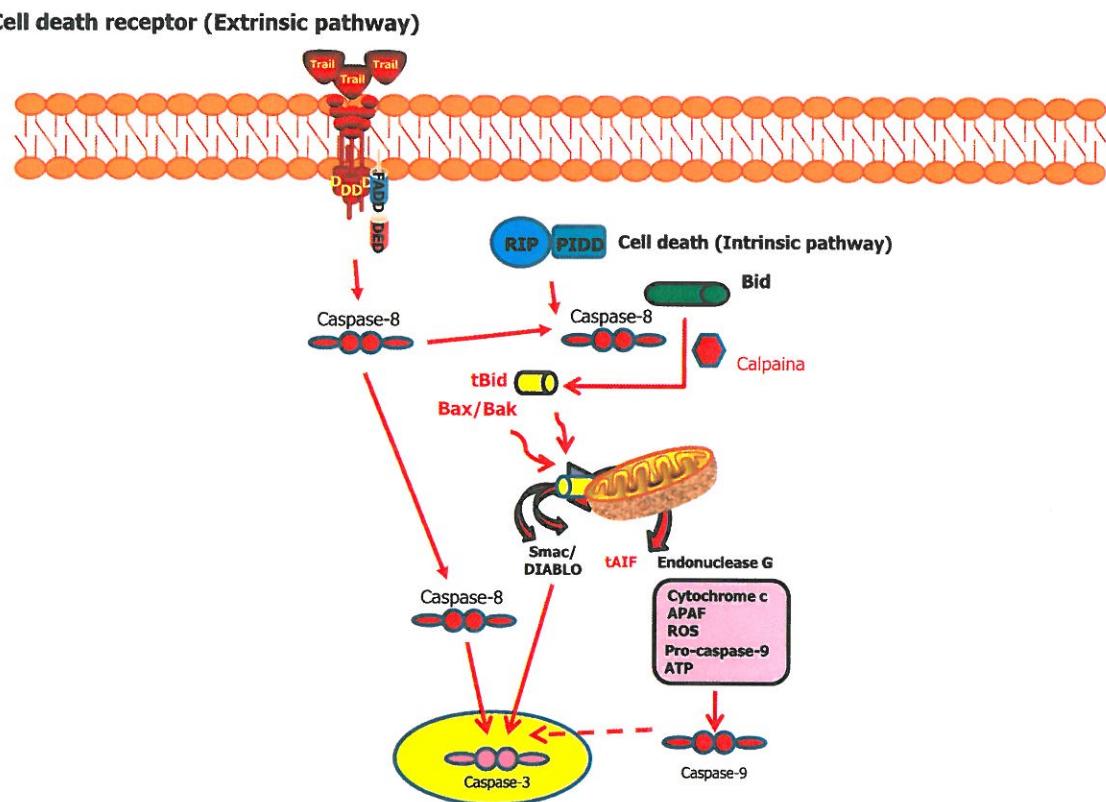




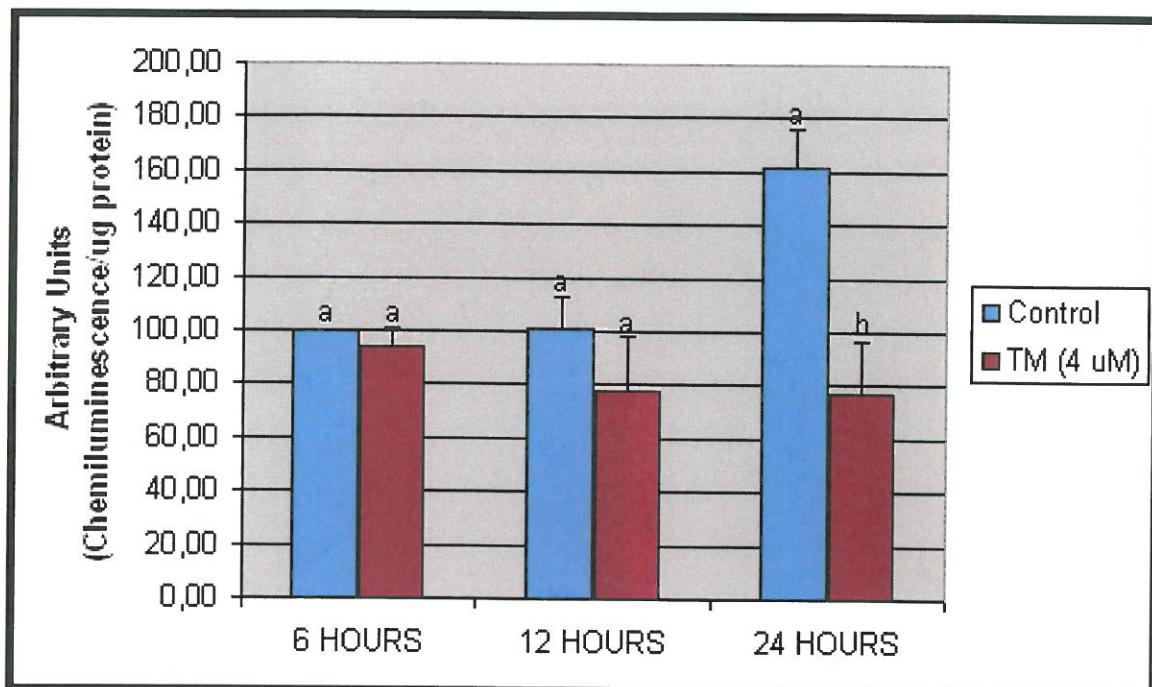
**Figure 3:** Regulation of ROS production by TM in fibroblast

## 2) REGULATION OF CELL DEATH (APOPTOSIS)

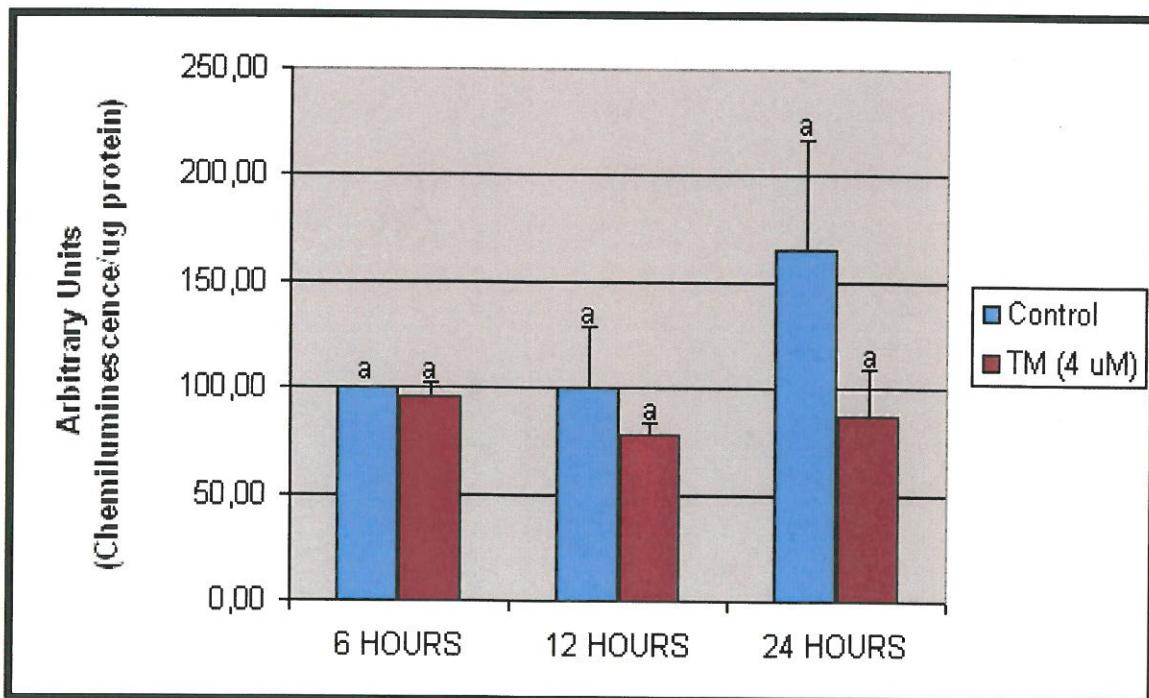
Cell death may be induced through extrinsic and intrinsic pathways as described below:



The measurement of caspase-3 is a suitable marked downstream marker of apoptosis. The measurement of caspase-3 in cell lysate is based on the use of commercial substrate which is specifically cleaved by caspase-3 generating a chemiluminescence signal (G8091, Promega).



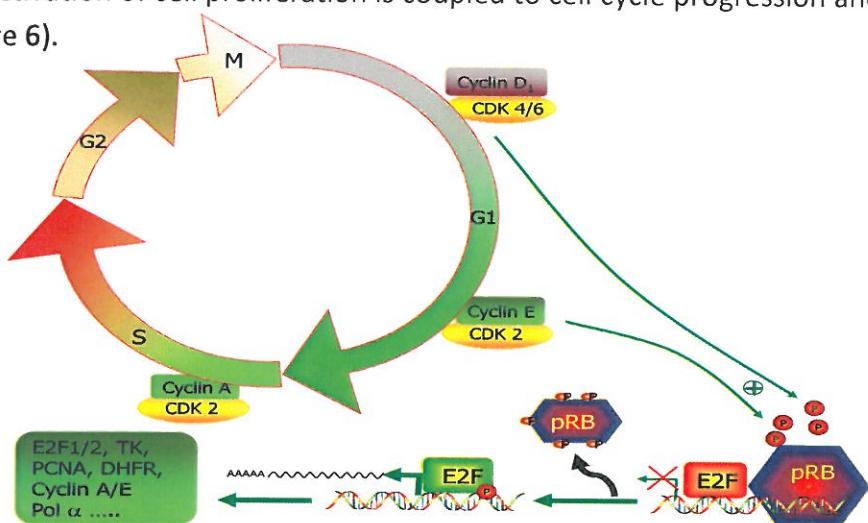
**Figure 4:** Regulation of caspase-3 activity by TM in fibroblast



**Figure 5:** Regulation of caspase-3 activity by TM in Keratinocytes

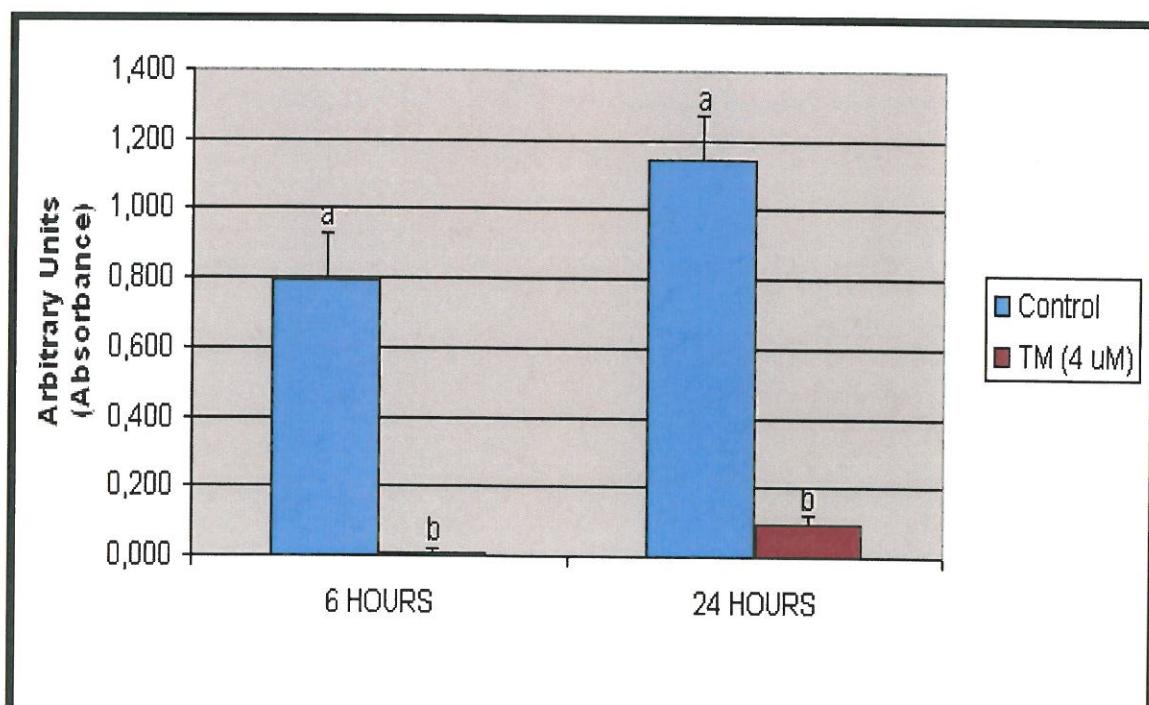
#### 4) REGULATION OF CELL PROLIFERATION

The activation of cell proliferation is coupled to cell cycle progression and DNA transcription (Figure 6).



**Figure 6:** Cell cycle scheme

The procedure used for the measurement of cell proliferation is based on the incorporation of bromo-deoxyuridine and further detection with specific antibodies (Cat N° 11669915001, Roche)



**Figure 7:** Regulation of cell proliferation by TM in fibroblasts.

## **CONCLUSIONS**

- 1) TM reduces superoxide anion production and increases nitric oxide generation dose dependently at short time point (1 hour) in fibroblasts.
- 2) Treatments do not change ROS production.
- 3) TM reduces apoptosis (24 hours) in fibroblasts and keratinocytes.
- 4) The anti-apoptotic effect of TM is related to a drastic reduction of cell proliferation (6 and 24 hours) in fibroblast.

## **SCIENTIFIC IMPACT**

Data enclosed in the present scientific report regarding the product developed by TERGUM S.L. named Tergum Maximum (TM) show that it has antioxidant properties towards reactive oxygen species (ROS) with associated increase in nitric oxide production. The biological significance of this fact may involve the potential vasodilatory properties of the compound. These beneficial properties are associated with cytoprotective activity characterized by reduction of apoptotic cell death in fibroblast and keratinocytes. Interestingly, TM strongly suppresses cell proliferation which could have a significant impact on cell repair and prevention of malignant cell processes.

We sign the report in Sevilla at November 11, 2014

Approved by,

Dr. Jordi Muntané Relat  
Main Investigator of the Agreement

Dr. José López Barneo  
Scientific Director of IBiS