



<LIFE98 ENV/B/000260>

**Detection and elimination of human exposure to  
environmental hormone disrupting substances.**

**Sertoli cell TM4 assay protocols**

<February 2003>

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| Laboratory for Andrology          | European Commission: Life98 fund     |
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| Laboratory of Hormonology         |                                      |
| Laboratory of Toxicology          |                                      |
| Hydraulics Laboratory             |                                      |

## **Experimental procedure TM4 cells.**

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*A detailed description of this procedure is in preparation, and will be presented for publication in a peer reviewed international journal.*

### **A Cell culture maintenance**

Stock cultures of TM4 cells (ATTC, Manassas, United States) were maintained in a 1:1 mixture of Ham's F12 nutrient mixture and Dulbecco's modified Eagle's medium supplemented with 365mg/L L-glutamine, 15mM HEPES, horse serum (5%), fetal bovine serum (2,5%), 100 units per mL penicilline and 100µg per mL streptomycinesulphate (all products Invitrogen N.V., Merelbeke, Belgium). Cells were grown in 25 cm<sup>2</sup> plastic tissue culture flasks (TTP, Novolab, Belgium) at 37°C and were subcultured every 5-7 days.

### **B Experimental culture procedure and growth experiments**

In preparation.

### **C Transient transfection of an ERE-Luc reporter plasmid in TM4 cells**

In preparation.