# Centre for AIDS Reagents.

## Data Sheet

**NAME:** 1G5/LTR – Luciferase cells

**REPOSITORY REFERENCE:** ARP5010

**CELL TYPE:** Jurkat derivative

**PROPAGATION MEDIUM:** RPMI 1640, 90%;
Foetal calf serum, 10%

**FREEZE MEDIUM:** RPMI 1640, 72.5%
Foetal calf serum, 20%
DMSO, 7.5%

**GROWTH CHARACTERISTICS:** 1G5 cells should be maintained at $<10^6$ cells/ml. Doubling time is approximately 24 hours. Split 1:10 every five days. The cells grow in suspension as single cells or small clumps. The suggested medium for 1G5 cells is RPMI with 10% fetal bovine serum; however, 1G5 cells have also been grown in RPMI with 5% fetal bovine serum and DMEM with 10% fetal bovine serum without appreciable change in their characteristics.

**MORPHOLOGY:** Characteristic T cell appearance; round, refractile with smooth edges.

**SPECIAL CHARACTERISTICS:** 1G5 is a Jurkat derivative containing a stably integrated HIV-LTR-luciferase construct. Cells were selected for low basal luciferase activity, HIV infectabilty, high responsiveness to *tat* expression, and high responsiveness to T-cell activation signals. Conditions can be established for quantitative analysis of LTR (HIV)-luciferase response to each of these conditions. A 10-1000-fold increase in luciferase activity can be achieved after transfection or infection of 1G5 with *tat*-expressing vectors or HIV. Equivalent levels of expression have also been detected after stimulation with T cell mitogens and stimulating environmental conditions. When used in conjunction with a *tat*-expressing vector, 1G5 provides a system for testing potential anti-*tat* therapies without the use of live HIV.



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**STORAGE:** Liquid Nitrogen

**SOURCE:** Dr Estuardo Aguilar-Cordova and Dr. John Belmont (courtesy of NIH AIDS Research and Reference Reagent Programme.)

**REFERENCE:** Aguilar-Cordova E, Chinen J, Donehower L, Lewis DE, Belmont JW. A sensitive reporter cell line for HIV-1 tat activity, HIV-1 inhibitors, and T cell activation effects. *AIDS Res Hum Retroviruses* **10**:295-301, 1994

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Please also ensure that you send us a copy of any papers resulting from work using reagents acquired through CFAR (this can be electronically or as a paper copy)

**NOTE:** Corporate requests should be directed to Dr. Aguilar-Cordova or Dr. Belmont, Baylor College of Medicine, Institute for Molecular Genetics, One Baylor Plaza, Houston, TX77030

## HIV-TAT DRUG INTERACTION STUDIES USING 1G5 CELLS

***Cell Culture and transfection:***

1G5 cells were maintained in RPMI 1640 supplemented with 10% HyClone fetal calf serum. Transfections were done in complete medium in a BTX electrporator at 120 V, 3000μF, in 125μl at 2.5 x $10^7$ cells/ml in a 0.2ml cuvette. Infections with retroviral vectors were done by supernatant exposure in complete medium containing 4.0 μg/ml polybrene. HIV infections using the NL4-3 laboratory strain were performed in complete medium. The infected cells were then treated with anti-viral compounds.

***Luciferase Analysis***

Five days after infection with HIV or a Tat retroviral vector (E. Aguilar-Cordova, unpublished data) and exposure to antiviral compounds, the cells were harvested, centrifuged at 1800 rpm for two minutes, washed once with PBS, and resuspended in 50 μl of luciferase lysis buffer [25 mM Tris-phosphate (ph 7.8), 2mM 1, 2-diaminocyclohexane-N,N,N',N'-tetraacetic acid, 10% glycerol, 1% Triton X-100]. The mixture was incubated at room temperature for 15-30 minutes and microfuged for 30 seconds. 10μl of the lysis supernatant was added to 50μl of luciferase assay reagent (Promega) and immediately read in a Packard scintillation counter set for single photon counting. All experiments were done in triplicate.