Cube Biosystems
A VitaScientific Brand

ViroVision™ HIV Reporter Cell System: Cell Culture Protocol

Important:

1) **Results are only guaranteed if ViroVision™ Infection Enhancement Medium is used when cells are infected with HIV.**

2) **Once thawed, the Infection Enhancement Medium should be stored at 4ºC, and is stable for 3 months. *Do not* re-freeze and *do not* leave *Infection Media* at room temperature.**

3) **Depending on the cell line, there may be different media requirements. Please consult the table at the end to match the proper media with purchased cell line.**

Contents

Item	QTY	Storage
ViroVision™ Cells	~ 5 X 10^6 Cells	Liquid N_2
ViroVision™ Infection Enhancement Medium	200 µl of 10X solution	-20ºC until needed. 4ºC for up to 3 months once thawed.

Initiation of ViroVision™ Cell Culture

1) Please ensure cells arrive frozen. Contact Cube Biosystems immediately if cells arrive thawed.
2) Store cells in liquid N_2 until needed.
3) To initiate cell culture, remove a vial of frozen cells from storage, and thaw as quickly as possible by placing in a 37°C water bath.

   Note: If cells are removed from liquid nitrogen, under sterile conditions, immediately unscrew cap ½ to 1 full turn to allow N_2 gas to escape. Re-secure cap. After cap is re-secured, place cells in 37ºC water bath to thaw.

4) Pre-warm 5 ml appropriate complete media in 15 ml tube to 37ºC. Add cells, dropwise, to pre-warmed media. Mix gently.
5) Collect the cells by centrifugation at 300 x g for 5 minutes, room temperature.
6) Remove/aspirate the supernatant and resuspend the cell pellet in 15 ml of complete media.
7) Place the cells into a T75 flask and incubate at 37°C, 5% CO_2.

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Culturing ViroVision™ Cells

1) Count cells daily and keep at a density below 1 x 10^6 cells ml^{-1}. Dead cells may be removed by Ficoll separation.
2) Add fresh complete media when cell density reaches 1 x 10^6 ml^{-1}.

HIV Infection

1) Count cells and pellet cells by centrifugation at 300 x g for 5 minutes.

   Note: Cell viability should be ≥ 80%.

2) Resuspend cells in complete media at concentration of ~ 2 x 10^6 cells ml^{-1}.
3) Use 100 µl of cells (~2 x 10^5) per infection.
4) Pre-treat cells by adding 10 µl of ViroVision™ Infection Enhancement Medium (10 x) so that Infection Media concentration is ~ 1 x. Mix and incubate for 2 hours. *Use of* ViroVision™ Infection Enhancement Medium *is required.*
5) Add virus to the cells & mix. Note volume of virus used.
6) Add Infection Enhancement Medium to 1/10 of the virus volume used. E.g. If 100 µl of virus used, add 10 µl of Infection Enhancement Medium. Incubate at 37°C for 2-4 hours.
7) Add 1 ml fresh complete media to wash cells. Pellet cells as above and remove supernatant. (Optional) Repeat 1 x for a total of 2 washes.
8) After washing, resuspend cells in 1 ml complete media.
9) Culture cells at 37°C, 5% CO_2 for 2-5 days. GFP or Luciferase may be quantified 48 hours after infection.

ViroVision™ Complete Media Guide

Cell Line	Media
Rev-A3R5	ViroVision™ Growth Media A: RPMI-1640 containing 10-15% FBS, 1% L-Glut, 1% Pen/Strep, and 1 mg/mL Geneticin (G418)
Rev-A3	ViroVision™ Growth Media B: RPMI-1640 containing 10-15% FBS, 1% L-Glut, 1% Pen/Strep
Rev-CEM	ViroVision™ Growth Media C: RPMI, 10% FBS