

## **Annex G1**

### **ICCVAM/NICEATM BG1Luc4E2 ER TA –Cell Viability Manual**

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**LUMI-CELL<sup>®</sup> ER ASSAY**  
**Visual Observation Cell Viability Manual**

**06 November 2010**

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## Procedure for Assessing Cell Viability Using the LUMI-CELL<sup>®</sup> ER Visual Observation Method

As per LUMI-CELL<sup>®</sup> ER assay agonist and antagonist protocols:

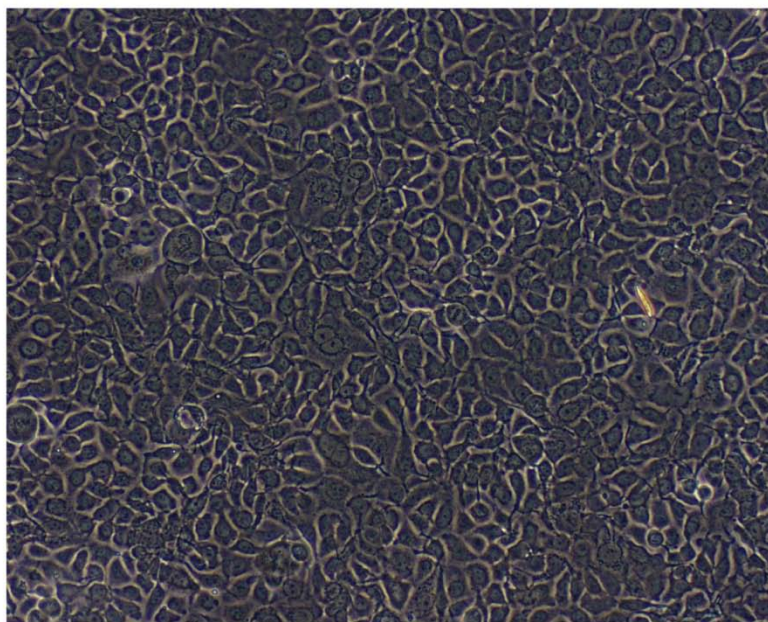
- Following nineteen to twenty four hours of incubation in test substances, remove the plates from the incubator and remove media by inverting the plate and lightly shaking over absorbent bench paper.
- Lightly tap the plate on the bench paper to remove excess liquid.
- Rinse the wells with 50 µl 1x PBS, and remove the PBS by inverting the plate and tapping it the plate on the bench paper to remove excess liquid.
- Examine all wells under an inverted microscope at 100X using phase contrast and assign cell viability scores for each well using the Visual Observation Scoring Table (**Table 1**) and **Figures 1 – 11** as reference.

**Table 1**                      **Visual Observation Scoring Table**

Viability Score	Brief Description <sup>1</sup>
1	Normal cell morphology and cell density
2	Altered cell morphology and/or small gaps between cells
3	Altered cell morphology and/or large gaps between cells
4	Few (or no) visible cells
P	Wells containing precipitation are to be noted with “P”

The following (**Figures 1-11**) are photomicrographs of individual wells from 96-well plates used in the LUMI-CELL<sup>®</sup> ER assay showing BG-1 cells observed through an inverted microscope at 100X using phase contrast after exposure to various substances. The scores were assigned using the visual inspection viability scoring system in **Table 1**.

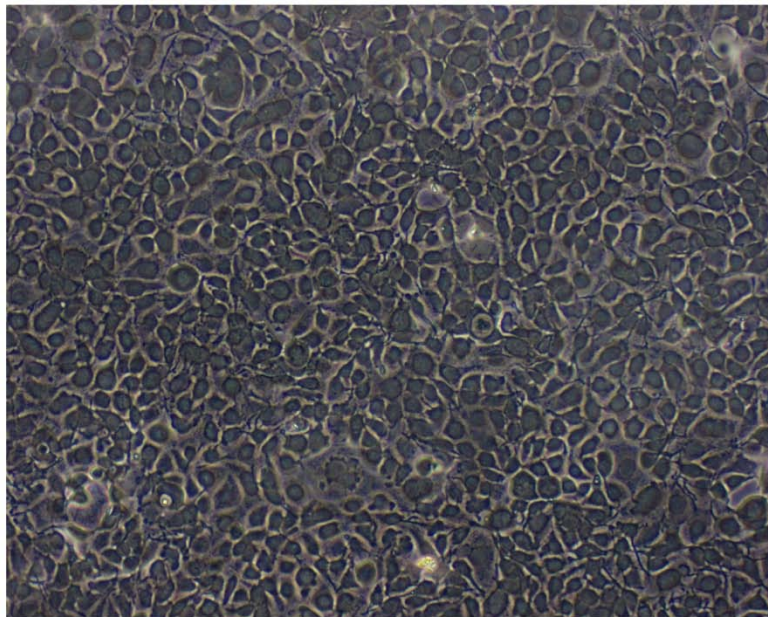
**Figure 1**      **Visual Observation Score of 1 (Example 1)**



No gaps  
between cells

Cells exhibit normal morphology in a monolayer with no gaps between cells.

**Figure 2**      **Visual Observation Score of 1 (Example 2)**

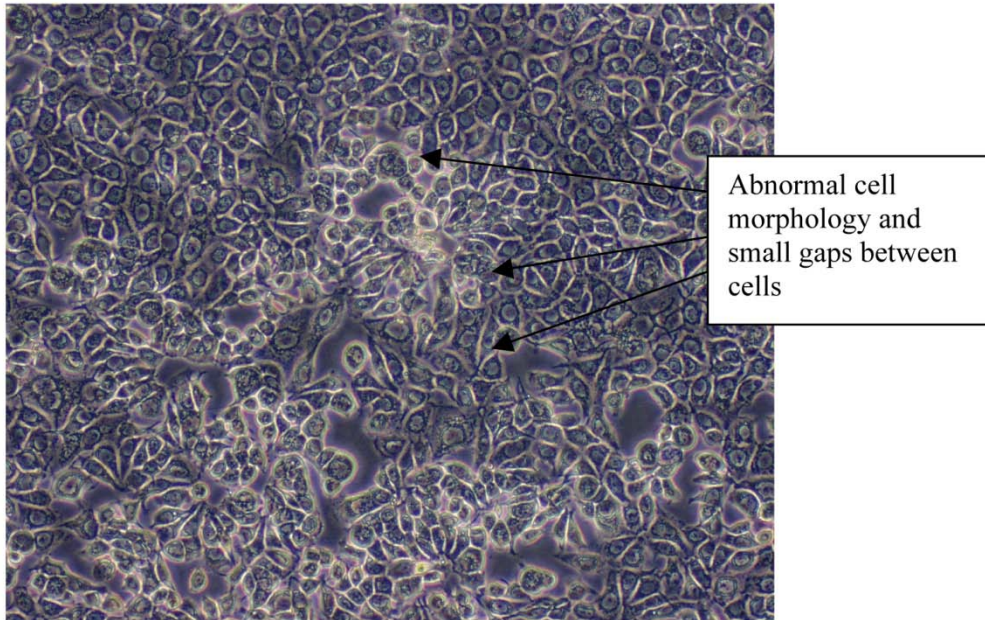


No gaps  
between cells

Cells exhibit normal morphology in a monolayer with no gaps between cells.

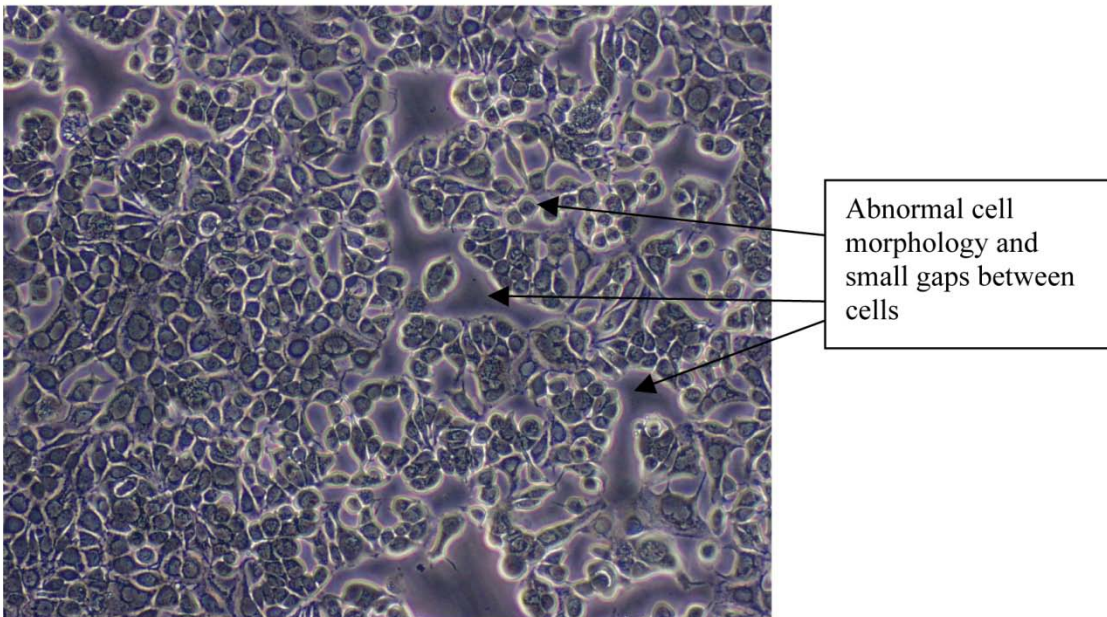


**Figure 3** Visual Observation Score of 2 (Example 1)



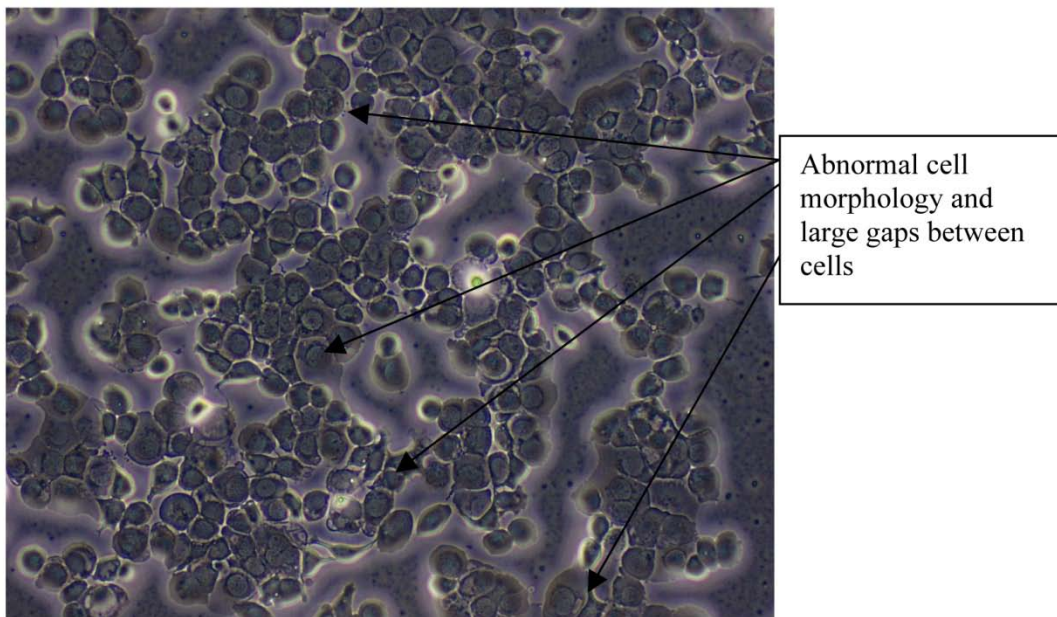
There are small gaps between cells, and some cells are abnormally rounded.

**Figure 4** Visual Observation Score of 2 (Example 2)



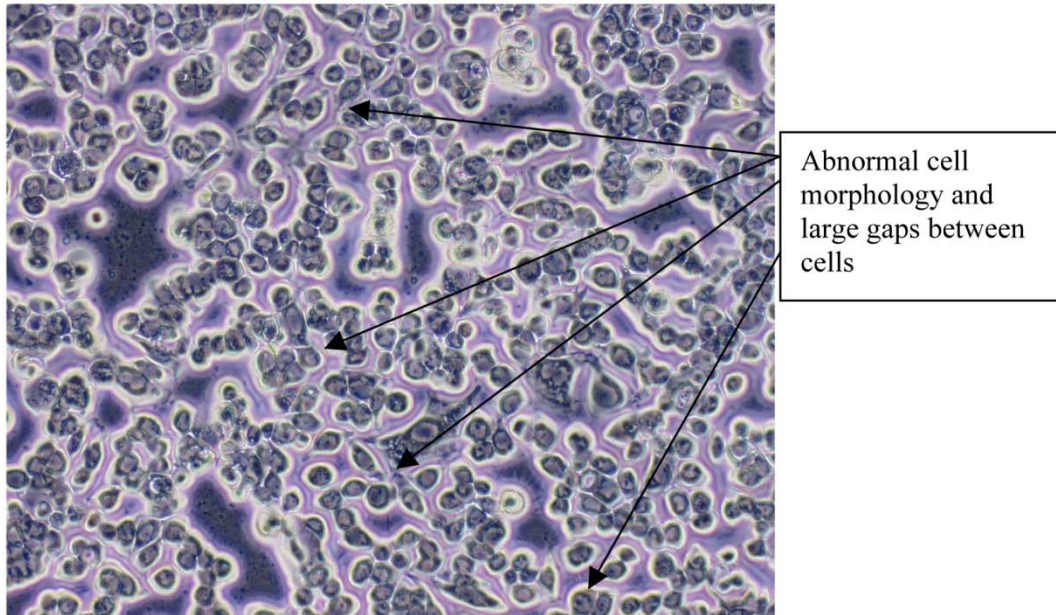
There are small gaps between cells, and some cells are abnormally rounded.

**Figure 5** Visual Observation Score of 3 (Example 1)



There are large gaps between cells and the majority of cells are abnormally rounded.

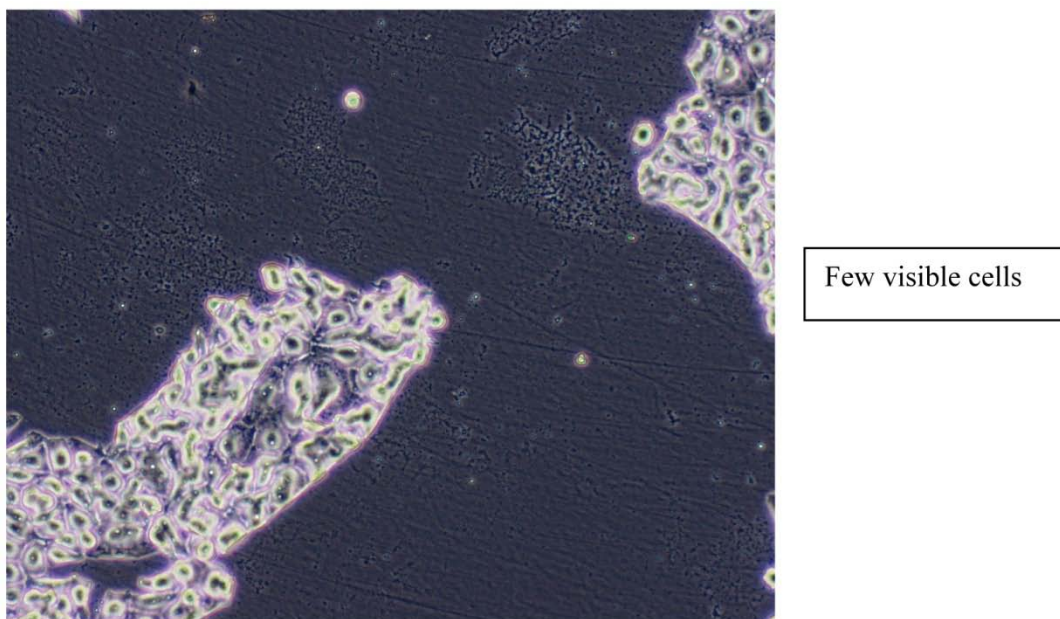
**Figure 6** Visual Observation Score of 3 (Example 2)



There are large gaps between cells and the majority of cells are abnormally rounded.



**Figure 7**      **Visual Observation Score of 4 (Example 1)**



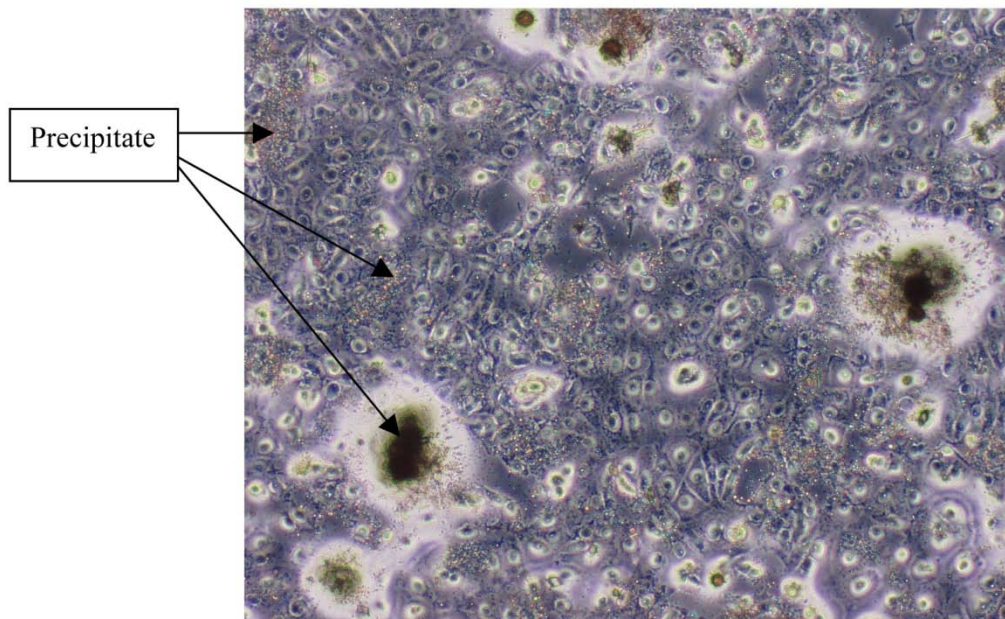
There are virtually no cells in the well.

**Figure 8**      **Visual Observation Score of 4 (Example 2)**

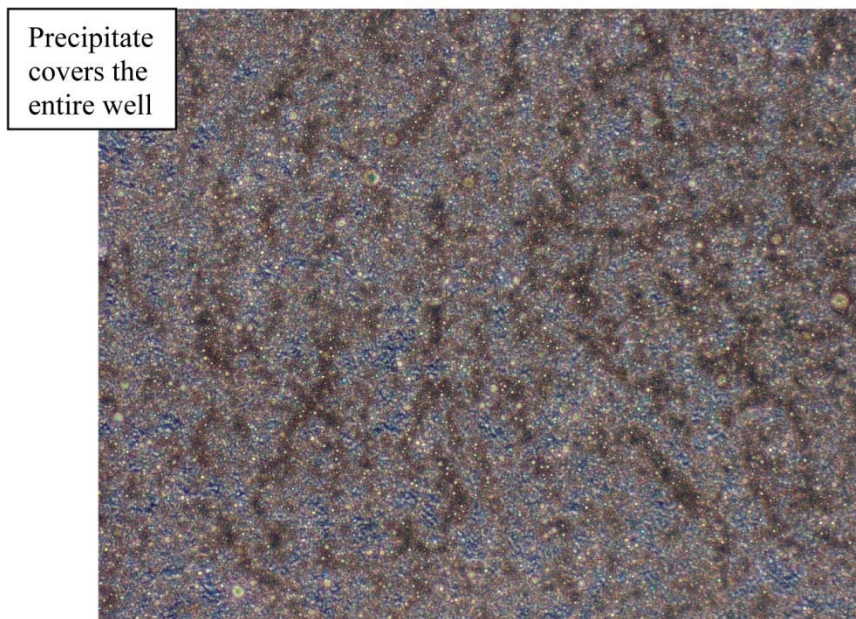


There are no visible cells in the well.

**Figure 9** Visual Observation noted with P for Precipitation (Example 1)



**Figure 10** Visual Observation noted with P for Precipitation (Example 2)



**Figure 11**      **Visual Observation noted with P for Precipitation (Example 3)**

