Product Information Sheet

MarkerGene™ Cell Proximity Assay Kit

Product M2751

Marker Gene Technologies, Inc.
University of Oregon Riverfront Research Park
1850 Millrace Drive
Eugene, Oregon 97403
1-888-218-4062
www.markergene.com
I. OVERVIEW

Cell-cell communication is an essential facet of growth and development for higher organisms. Understanding and measuring these cellular interactions presents an intricate challenge for both in vitro and in vivo study. Marker Gene has developed an innovative assay system that utilizes a proprietary luminescent substrate dependent upon interaction with two enzymes in order to generate light. The light from successful turnover of the substrate is emitted at a red wavelength (λ=619) and the intensity of this light output can quantitatively be measured as an indication of cell proximity and interaction. The more communication that occurs between the two enzyme-producing cells or the closer they are in proximity to one another, the more intense the light output.

Our reporter assay system provides a non-invasive, non-lethal method of identifying intricate cellular communications, with a multitude of potential in vivo applications. These applications can range from tumor detection and treatment monitoring, to examining the mechanics of immune system responses, or even measurement of other enzyme-substrate interactions.

The assay utilizes each enzyme as a separate vector that is transfected into a cell line which can then be combined or added to a plate, gel matrix, tissue, or organism for analysis. Due to the enzyme and substrate permeability, there is no lysis of the cells before addition of the substrate and measurement of luminescence, making it ideal for live-cell and whole-organism imaging. We have demonstrated the principle of the assay in cultured cells, which produce light only when both transfected cell lines expressing the vectors are in the presence of the substrate. The light signal emitted is also cell number dependent and will change with varying densities of each transfected cell line.

The high copy number eukaryotic vector, pDC57 expresses a mutant luciferase gene under the control of the cytomegalovirus immediate early gene promoter (CMV IE). This vector encodes a mutant enzyme that catalyzes the production of long wavelength (red) light (Em: 605) from D-Luciferin. The mutant luciferase is optimized for expression in mammalian cells, resulting in drastically improved light production, allowing detection of expression at extremely low levels. The mutant luciferase has also been shown to remain stable at 37°C over long time periods, a characteristic not seen in
wild type luciferases. This vector is not only useful for transfection of mammalian cells in culture, but may also be used in other species. For more information on this vector, refer to product M1394 on our website www.markergene.com.

The pCMVβ vector contains a full length β-galactosidase gene which is codon-optimized to increase expression by 15-fold compared to the native E. coli gene sequence. This vector also has enhanced transcript stability and increased translation efficiency. For more information, refer to product M1017 on our website at www.markergene.com.
II. PROTOCOL

Methods of Transfection:

For established cell lines (e.g. HeLa, 293, CHO, etc.), consult original references or the supplier of your cell line for the optimal method of transfection. Pay particular attention to the cell medium requirements (serum or serum-free), when to pass the cells, the ideal confluency for transfection, etc. Further information is provided in *Current Protocols in Molecular Biology* (Ausubel et al., 1994).

Methods for transfection can include Calcium Phosphate, Lipid-mediated, Electroporation, or Cationic Polymers. Refer to additional information for recommended brands/kits for each transfection method.
Cellular Assay Procedure:

1. Resuspend each lyophilized vector in 20 μl sterile DI water to prepare 1μg/μL stock solutions and store at -20°C.

2. Dissolve Substrate in Reaction Buffer to a concentration of 1.5 mg/ml and store at -20°C.

3. Seed the cell line to be transfected at the recommended density for the selected transfection method, and incubate overnight.

4. Transfect separately growing wells or plates with each of the vectors provided: the β-Galactosidase Vector and the Red Luciferase Vector. Follow the same protocol of the selected transfection method for both vectors. Incubate transfected cells overnight.

5. Harvest transfected cells. Seed a 96-well microtiter plate with 1·10⁴ cells of each transfected cell line per well, to a final density of 2·10⁵ cells/well. Mix well, and incubate overnight.

   **Note:** if possible, seed enough wells for each desired test condition/concentration in triplicate, and include separate controls for each cell line.

   i.e. If both harvested transfected cell lines are at a density of 2·10⁵ cells/mL, mix 50μL of each cell line in a single well (100μL total).

6. Treat seeded cells with desired compound/conditions.

7. Aspirate media from incubated cells and replace with 50μL fresh media at least 1 hour before adding substrate.

8. Add 50μL of Substrate to each well and measure luminescence on a plate reader. It may take up to 15 minutes for the enzyme/substrate reaction to produce a reliable luminescent signal. There should be no luminescent signal from only Luciferase cells or only β-Galactosidase cells.
HeLa cells were transfected with the Red Luciferase and β-Galactosidase vectors using the jetPRIME cationic polymer method. The following day, the cells were harvested and reseeded at a density of 2×10⁴ cells/well, following the protocol above. (For "Mixed cells" a 1:1 concentration of Luciferase cells to β-Galactosidase cells was seeded). 50μL of either M2751 Substrate, or D-Luciferin in the same buffer was added to each well and their luminescence measured on a Tecan Infinite M200Pro.

Additional Information:

Recommended brands for transfection methods:

- Calcium Phosphate: ThermoFisher
- Lipid-mediated: Lipofectamine from GIBCO-BRL
- Electroporation: Bio-Rad
- Cationic Polymers: jetPRIME from Polyplus Transfection
III. TROUBLESHOOTING

If the luminescent signal of just the luciferase cells is too high:

- The cells being used for the transfection/assay may have a high endogenous level of β-galactosidase. It is recommended that the background level of enzyme activity be measured prior to conducting the assay. Please refer to products M0255 or M0259 for our lacZ β-galactosidase fluorescent detection kits.

If the luminescent signal of the transfected cells mixed together is too low:

- The transfection efficiency may also be low. It is recommended that a reporter vector, such as a GFP expression vector, is transfected in parallel with the kit’s vectors to ensure transfection efficiency.
- The viability of the transfected cells may be low.
- The cells may no longer be expressing the transiently transfected vectors if the user waits too long before measuring luminescence. Refer to the used transfection protocol for the optimal timeframe of gene expression. This is generally within 48 hours after transfection.

<table>
<thead>
<tr>
<th>M2751 Kit Contents</th>
<th>DESCRIPTION</th>
<th>QUANTITY</th>
<th>PART NO.</th>
<th>STORAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>REAGENTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Red Luciferase Vector</td>
<td>1 x 20 µg</td>
<td>2751-001</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>2 β-Galactosidase Vector</td>
<td>1 x 20 µg</td>
<td>2751-002</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>3 Substrate</td>
<td>1 x 5 mg</td>
<td>2751-003</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>4 Reaction Buffer</td>
<td>1 x 5 mL</td>
<td>2751-004</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td><strong>DOCUMENTATION</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product Information Sheets</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: F=store at or below -20°C; C=store cold (4°C) Read protocol instructions carefully prior to use.
IV. REFERENCES

NOTES:
CONTACT AND SUPPORT

For questions or comments on this or any product from Marker Gene Technologies, Inc., you may contact us by phone or via our website. We welcome customer feedback and we make every effort to improve our products based on input from our clients.

To ask a question or make a comment or suggestion, you can call us at 1-888-218-4062 or fax to 541-342-1960.

For more information on our products and services, please visit our website at www.markergene.com, where you can find:

- Secure online ordering
- Product Information
- MGT Scientific Newsletters
- Corporate Information
- Custom Synthesis Info

We want to thank you for your purchase and hope that you will continue to order from Marker Gene Technologies, Inc.

Marker Gene Technologies, Inc.
University of Oregon Riverfront Research Park
1850 Millrace Drive
Eugene, Oregon 97403
1-888-218-4062
www.markergene.com