**In Vivo Luciferase Imaging Kit**

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**Catalog No.** 631758  
**Amount** 2.2 mg  
**Lot Number** Specified on product label.

**Product Information**

The *In Vivo Luciferase Imaging Kit* provides a bioluminescent, cell-based assay that simplifies the analysis of promoter function. When a functional promoter is cloned into the pMetLuc-Mem Reporter vector, a luciferase-based reporter protein is expressed and displayed on the cell surface. This membrane-bound luciferase is easily visualized by adding substrate to the cell medium. The assay produces > 20-fold higher signal than similar assays involving (cytosolic) firefly luciferase. The Kit can also be used for whole-animal imaging applications. The Kit contains pMetLuc-Mem Reporter and Control vectors and a lyophilized substrate.

**Package Contents**

- 20 µl pMetLuc-Mem Reporter Vector (500 ng/µl)
- 20 µl pMetLuc-Mem Control Vector (500 ng/µl)
- 4 tubes Lyophilized Secreted Luciferase Substrate (550 µg/tube)

**Storage Conditions**

- Store at −20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

**Shelf Life**

- 1 year from date of receipt under proper storage conditions.

**Shipping Conditions**

- Domestic: Dry ice (−70°C)
- International: Dry ice (−70°C)

**Product User Manuals**

User manuals for Clontech products are available for download at [www.clontech.com/manuals](http://www.clontech.com/manuals)

The following user manual applies to this product:

- *In Vivo Luciferase Imaging Kit Protocol (PT5160-2)*
**pMetLuc-Mem Reporter and Control Vector Information**

**Figure 1.** pMetLuc-Mem Reporter and Control Vector Maps.

**Figure 2.** pMetLuc-Mem Reporter Vector Multiple Cloning Site (MCS).

**Description**

pMetLuc-Mem Reporter is a promoterless reporter vector that simplifies the analysis of promoter function in live cells. Insertion of a functional promoter into the vector’s multiple cloning site (MCS) causes the expression of TCA-TFR/MetLuc, a membrane-bound, luciferase-based reporter protein that is displayed on the cell surface. TCA-TFR/MetLuc is a chimeric protein made up of an N-terminally truncated human transferrin receptor (TCA-TFR; a type II transmembrane protein; 1) that is C-terminally fused to a mutant form of Metridia luciferase, a normally secreted protein from the marine copepod *Metridia longa* (2), that is missing the N-terminal signal peptide necessary for secretion. The truncated transferrin receptor contains a signal/anchor domain for ER and membrane targeting, but lacks the internalization signal and protein kinase C phosphorylation site present in the full-length receptor (1). The resulting chimeric protein is anchored in the cell membrane in such a way that the luciferase is accessible from the extracellular environment.
SV40 polyadenylation signals downstream of the TCA-TFR/MetLuc gene direct proper processing of the 3' end of the chimeric mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in E. coli, and an f1 origin for single-stranded DNA production. A neomycin resistance cassette (Neo') allows stably transfected eukaryotic cells to be selected using G418. This cassette consists of the SV40 early promoter, the Tn5 kanamycin/neomycin resistance gene, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene. The vector also contains a synthetic transcription blocker (TB), composed of adjacent polyadenylation and transcription pause sites, that reduces background read-through transcription (3). A bacterial promoter upstream of the cassette allows kanamycin resistance in E. coli.

pMetLuc-Mem Control is a control vector that constitutively expresses the chimeric luciferase TCA-TFR/MetLuc from the human cytomegalovirus immediate early promoter (\(P_{\text{CMV IE}}\)). When used with standard luciferase detection reagents, this vector can be used as a reporter of induction efficiency. pMetLuc-Mem Control is not intended to be used as a cloning vector.

**Location of Features**

**pMetLuc-Mem Reporter Vector**
- MCS (multiple cloning site): 12–83
- TCA-TFR/MetLuc (chimeric non-secreted, membrane-bound *Metridia* luciferase): 97–927
- SV40 early polyA signals: 1082–1116
- f1 origin of replication: 1179–1634 (complementary)
- SV40 origin of replication: 1975–2113
- Kan'/Neo' (kanamycin/neomycin resistance gene): 2159–2950
- HSV TK polyA signal: 3189–3207
- pUC origin of replication: 3538–4181
- TB (transcription blocker): 4211–4364

**pMetLuc-Mem Control Vector**
- \(P_{\text{CMV IE}}\) (human cytomegalovirus immediate early promoter): 1–589
- TCA-TFR/MetLuc (chimeric non-secreted, membrane-bound *Metridia* luciferase): 597–1427
- SV40 early polyA signals: 1583–1617
- f1 origin of replication: 1680–2135 (complementary)
- SV40 origin of replication: 2476–2614
- Kan'/Neo' (kanamycin/neomycin resistance gene): 2660–3454
- HSV TK polyA signal: 3690–3708
- pUC origin of replication: 4039–4682

**Additional Information**

Upon induction, functional promoters will drive the expression of the membrane-targeted luciferase, while nonfunctional promoters will not. The presence of the luciferase can be easily detected by adding luciferase substrate to live cells and analyzing the sample in a luminometer. Promoter function can be quantified by the relative intensity of the bioluminescent signal. The vector can also be used for live animal imaging applications.

The vectors can be transfected into mammalian cells using any standard transfection method. Stable transfectants can be selected using G418 when required (4).
**Certificate of Analysis**

*In Vivo* Luciferase Imaging Kit

**Cat. No. 631758**

**Propagation in E. coli**

- Recommended host strain: DH5α™, XL1 Blue, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 μg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

**References**


**Quality Control Data**

**Plasmid Identity & Purity**

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

<table>
<thead>
<tr>
<th>Vector</th>
<th>Enzymes</th>
<th>Fragment Sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMetLuc-Mem Reporter</td>
<td>AfeI</td>
<td>4.4 kb</td>
</tr>
<tr>
<td></td>
<td>XmaI</td>
<td>0.5 &amp; 3.9 kb</td>
</tr>
<tr>
<td>pMetLuc-Mem Control</td>
<td>PmlI</td>
<td>4.8 kb</td>
</tr>
<tr>
<td></td>
<td>NdeI &amp; XmaI</td>
<td>0.8 &amp; 3.9 kb</td>
</tr>
</tbody>
</table>

- Vector identity was confirmed by sequencing.
- $A_{260}/A_{280}$: 1.8–2.0

**Substrate Identity**

- The chemical identity of the substrate was confirmed by NMR analysis.

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This document has been reviewed and approved by the Clontech Quality Assurance Department.