ACCEGEN
BIOTECHNOLOGY

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AcceGen

Reporter Stable Cell Line Product Manual

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1. Description

Reporter stable cell lines are widely used for detecting target gene expression, studying protein function and localization, and measuring protein production. They enable real-time visualization of target gene activity, allowing researchers to monitor protein synthesis and intracellular distribution in living cells. In addition, reporter genes allow continuous monitoring of endogenous or recombinant protein expression levels. These cell lines can also be applied to investigate the transcriptional activity of gene promoters.

In reporter cell lines, a reporter gene is introduced into target cells through genetic engineering techniques to achieve stable expression. This allows researchers to track the localization and expression of target genes within the cells. Lentiviral transduction is most commonly used for this purpose. Typical reporter genes include GFP, RFP, YFP, luciferase, and others. Based on customer needs, we provide customized development services for dual-luciferase reporter systems and various other types of functional reporter gene cell lines.

With our customized service, you can benefit from a high-quality and efficient development experience.

2. Reporter Stable Cell Line Generation Service

Service	Technical method	Gene expression evaluation	Type	Delivery
Reporter Stable Cell Line Generation	CRISPR/Cas9	1. qPCR 2. Function verification	1. Common Reporter Cell Line (Luciferase/GFP/RFP…) 2. Functional reporter Cell Line such as pathways and targets	Cell pool / Monoclonal cell line

3. Technical advantages

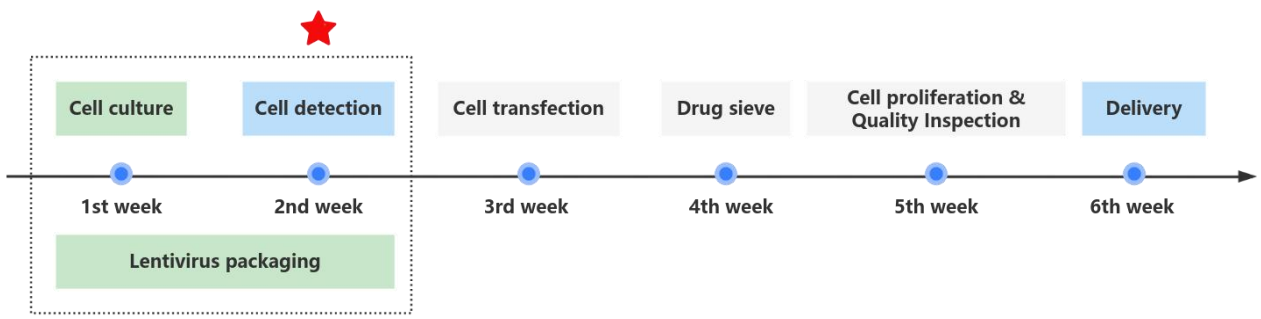
For Common Reporter Cell Line, our lentiviral vector has high infection efficiency and enable rapid integration of the target gene into the host cell genome. This significantly enhances gene expression, allowing long-term and stable expression. We offer a wide selection of reporter genes and host cell types for customers to choose from. In addition, we provide services such as cell gene expression regulation and cellular function verification. Finally, professional laboratory and quality control reports will be delivered.

We possess a strong track record in the custom construction of functional reporter cell lines. Our experience spans diverse targets and pathways, including but not limited to key signaling nodes like Nf κB and cytokine receptors such as IL-5. We are equipped to develop robust models to illuminate your specific biological questions.

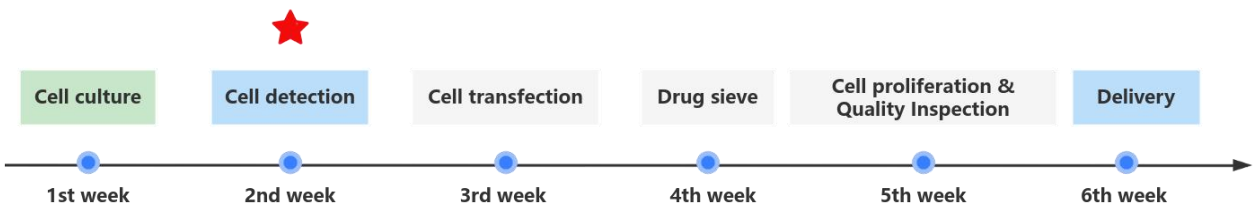
4. Experimental process

4.1 Common Reporter Cell Line (Luciferase/GFP/RFP/mCherry, etc.)

(1)Lentivirus transfection method

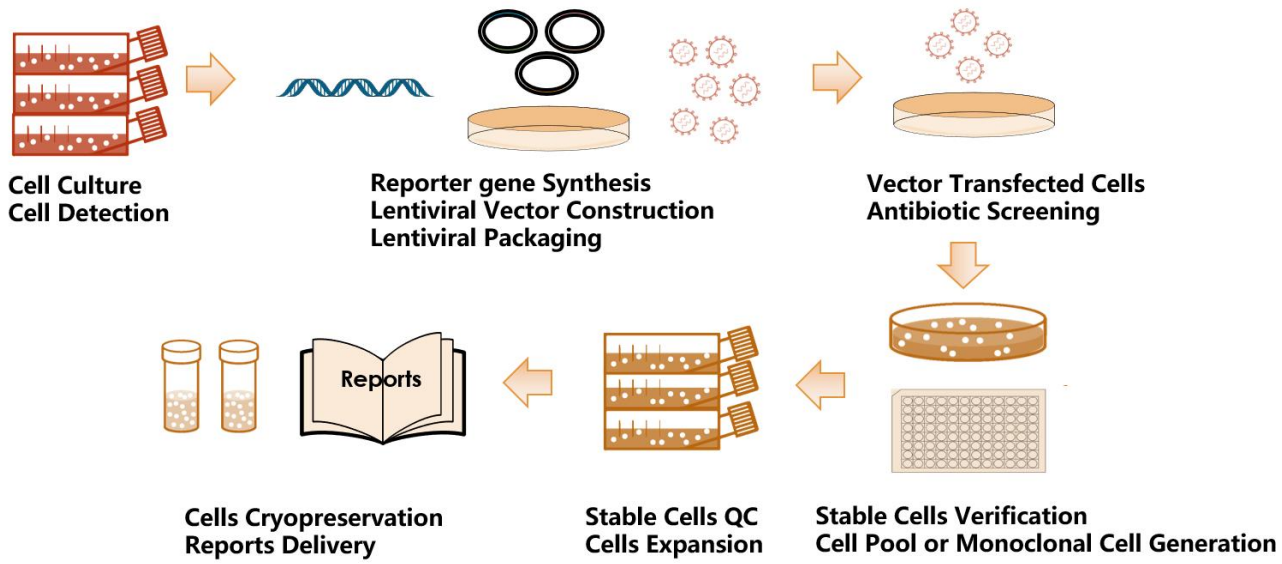


(2)Electroporation method or Lipo transfection method



4.2 Functional Reporter Cell Line

Cell Culture and Detection (2-3 weeks)	Vector Construction and Lentivirus packaging (3-5weeks)	Lentivirus-mediated cell transduction (3-6 weeks)	Stable cell line QC (1 week)
• Bacteria and mycoplasma detection • The genotype of the target cells detection	• Reporter gene synthesis; • Lentiviral vector construction • Lentiviral packaging and lentiviral titer determination	• Drug-resistance selection • Reporter cell generation(cell pool or monoclonal cell)	• QPCR & stability test • Cell lines and reports Delivery



Note: *The displayed timeline is for reference only. The specific lead time depends on the project assessment.*

Our Commitment and Important Considerations: *Our service guarantees the precise and accurate construction of your custom reporter system, ensuring the genetic elements are integrated as designed.*

It is important to note that the ultimate functional performance of the reporter line - such as the magnitude of signal response - is influenced by inherent biological complexity and requires a phase of dedicated post-construction screening and validation. Therefore, within the project cycle, it is impossible to guarantee 100% functionality.

5. Application Operations and Detection Methods

5.1 Target gene function discovery and research

a) Construct a gene reporter stable cell line

b) Culture and expand the reporter stable cell line using the suitable medium and serum

c) Group cells according to experimental requirements

d) Study the expression level of genes on cells or in vivo tracking through various experimental methods in terms of phenotype or gene function.

5.2 Default verification services

RT-qPCR: Cellular RNA is extracted using a nucleic acid extraction kit, and sDNA is obtained by reverse transcription. SYBR Green or TaqMan Probe methods can be used to detect the expression level of the reporter gene or target gene.

5.3 Value-added services

(1) **Western blotting:** Cells are lysed using RIPA or other reagents, and the cell supernatant is collected after centrifugation to determine the concentration of the target protein. The difference in protein expression between reporter stable cells and control cells can be obtained.

(2) **Flow Cytometry:** Inoculate cells (5×10^5 cells/mL) into 6-well plates and culture them for 24 hours. Add antibodies or stimulating factors required for the experiment to the cells and incubate for several hours. Finally, a flow cytometer can be used to detect cell cycle and apoptosis.

(3) Function verification (Optional): We can perform tailored assays by stimulating your cells with relevant stimuli (e.g., ligands, cytokines) to directly quantify the reporter response—measuring induction, kinetics, and potency. This critical step goes beyond genetic construction, providing definitive evidence that your reporter cell line is biologically responsive and ready to accelerate your research in target validation and drug screening.

6. Delivery of Cell Lines and Report

1 vial of stable cell line (≥ 0.5×10^6 cells/vial) and customized project report will be delivered to the customer. If the project requires the customer to provide a host cell, we will request the customer to fill in AcceGen Custom Stable Cell Line Service Requisition Form.

7. Experimental case report

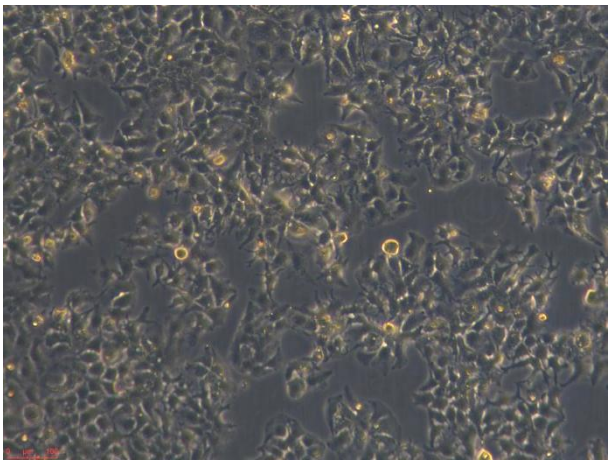
7.1 Common Repoter Cell Line

Case 1: Huh-7 GFP Reporter Cell Line (PB Transpose System)

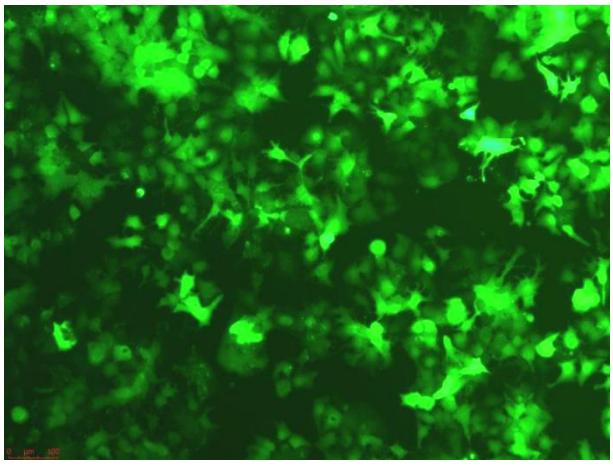
Experimental Overview:

A stable monoclonal cell line was constructed through the following key steps: First, well-cultured target cells in good condition were transfected, and GFP expression was confirmed by fluorescence microscopy. Subsequently, the cells were subjected to puromycin for at least 48 hours. Following selection, surviving cells were expanded in medium containing a maintenance concentration of puromycin. During the expansion phase, a portion of the cells was collected for PCR analysis. The PCR-positive mixed population was then seeded into 96-well plates for monoclonal isolation. Individual clones were further screened by PCR, and confirmed positive clones were expanded and cryopreserved, completing the construction of the stable monoclonal cell line.

The morphology of positive clones is shown below (100X magnification):



Huh-7 GFP bright field



Huh-7 GFP fluorescence

Case 2: T2 Luciferase Reporter Cell Line (Lentivirus)

Project Description:

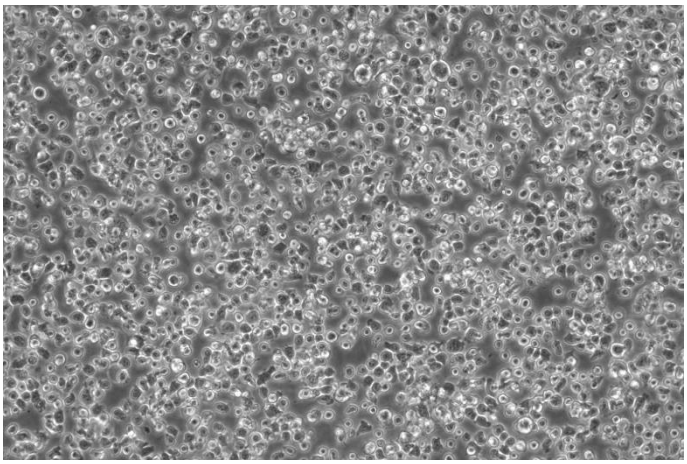
Generation of T2 Stable Cell Line for Luciferase fusion protein Overexpression with Lentivirus.

Generation of the Over-expression cell lines

T2 cells have been transduced with lentivirus particles below:

pLV[Exp]-Puro-EF1A>Luciferase

The morphology of positive clones is shown below (100X):



Detection of the luciferase enzyme

The T2 # Luciferase # Poolcells luciferase luminescence value was 50000 fold higher thanthe T2 WT.

Sample name	Parallel 1	Parallel 2	Parallel 3	Mean	Expression multiple
T2 Luciferase	1010000	1020000	1000000	1010000	50000
T2 WT	20.2	20.1	20.5	20.2	

Case 3: B16-OVA Luciferase Reporter Cell Line

Project Description:

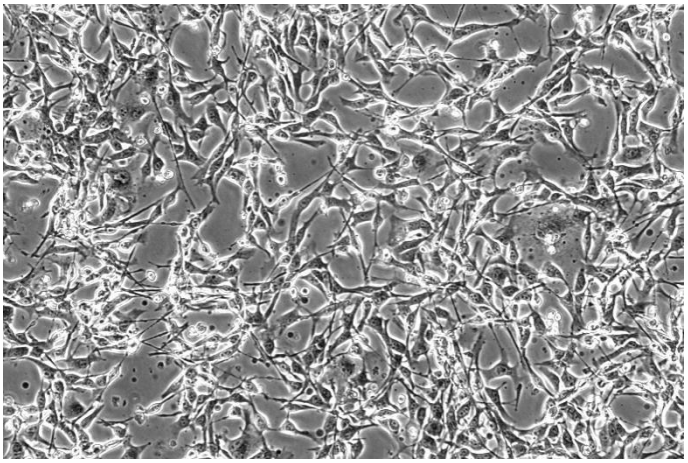
Generation of B16-F10 Stable Cell Line for Luciferase fusion protein Over-expression with Lentivirus.

Generation of the Over-expression cell lines

B16-F10 cells have been transduced with lentivirus particles below:

pLV[Exp]-CMV>ggaOVAL[NM 205152.3]-EF1A>Puro:T2A:Luciferase

The morphology of positive clones is shown below (100X):



Validation of the lentivirus insertion and GOI expression

RT-qPCR

RT-qPCR was performed on total RNA extracted from the three samples:

B16-F10#OVAL&Luc#Poolcells, wildtype B16-F10 cells and ddH_2O.

Primer information

Gene	Primer	Primer Sequence (5'->3')	Length (bp)
OVAL (gene of interest)	F	ATTCGTTCAGCCTTGCCAGT	215
	R	AGAATCCACGGAGCTTGGC	

Result

Group	OVAL	mGAPDH	Δ CT
B16-F10#OVAL&Luc	21.52	15.98	5.54
B16-F10	Undetectable	16.36	N/A
H_2O	Undetectable	Undetectable	N/A

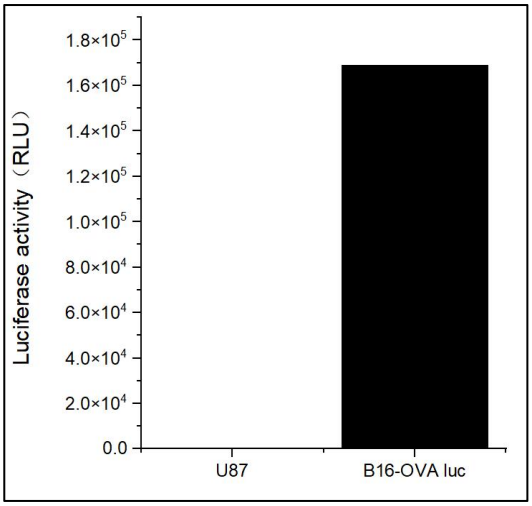
Conclusion

The RT-qPCR results show that OVAL mRNA is efficiently expressed in B16-F10#OVAL&Luc#Poolcells.

Detection of the luciferase enzyme

The B16-F10#OVAL&Luc luciferase luminescence value was 1123.5765 fold higher thanthe B16-F10 WT.

Sample name	Parallel 1	Parallel 2	Parallel 3	Mean	Expression multiple
B16-F10#OVAL&Luc	171638	165880	169215	168911	1123.5765
B16-F10 WT	170	167	114	150.333333	



Case 4: B16-F10 Luciferase tdTomato Reporter Cell Line

Project Description:

Construction of B16-F10 cell line with simultaneous over-expression of Luciferase and tdTomato fluorescence with Lentivirus.

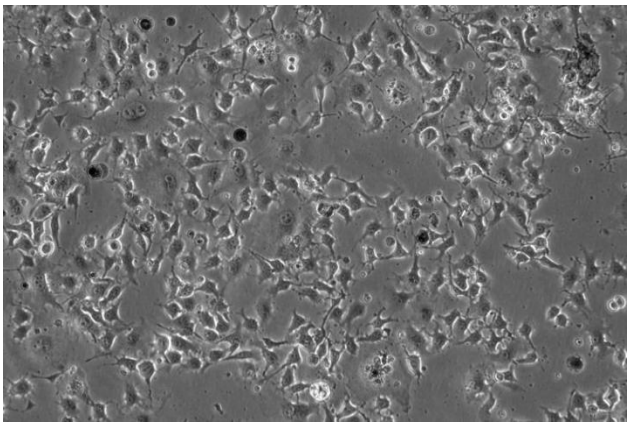
Generation of the Over-expression cell lines

B16-F10 cells have been transduced with lentivirus particles below:

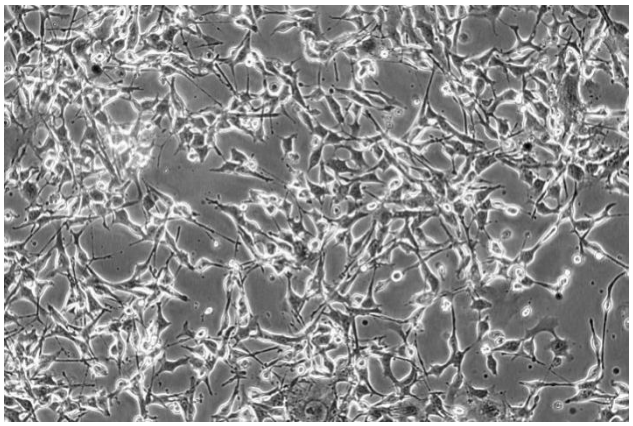
pLV[Exp]-CMV>Luciferase-EF1A>tdTomato:T2A:Puro

pLV[Exp]-CMV>MCS-EF1A>Puro-WPRE

The morphology of positive clones is shown below (100X):



B16-F10#Luc&tdTomato#Poolcells



B16-F10#Control#Poolcells

Validation of the lentivirus insertion and GOI expression

RT-qPCR

RT-qPCR was performed on total RNA extracted from the three samples:

B16-F10#Luc&tdTomato#Poolcells, wildtype B16-F10 cells and ddH_2O.

Primer information

Gene	Primer	Primer Sequence (5'->3')	Length (bp)
Luciferase (gene of interest)	F	GTCACATCTCATCTACCTCCCG	238
	R	AACCGTGATGGAATGGAACAAC	
tdTomato (gene of interest)	F	TACATGGCCAAGAAGCCCGT	156
	R	GTACAGCTCGTCCATGCCG	

Result

Group	Luc	mGAPDH	Δ CT
B16-F10#Luc&tdTomato	20.06	14.14	5.92
B16-F10	Undetectable	13.86	N/A
H_2O	Undetectable	Undetectable	N/A

Group	tdTomato	mGAPDH	Δ CT
B16-F10#Luc&tdTomato	19.43	14.14	5.29
B16-F10	Undetectable	13.86	N/A
H_2O	Undetectable	Undetectable	N/A

Conclusion

The RT-qPCR results show that Luc&tdTomato mRNA is efficiently expressed in B16-F10#Luc&tdTomato#Poolcells.

7.2 Functional Reporter Cell Line

Case 1: NK-92MI (NFkB) Luciferase Reporter Cell Line

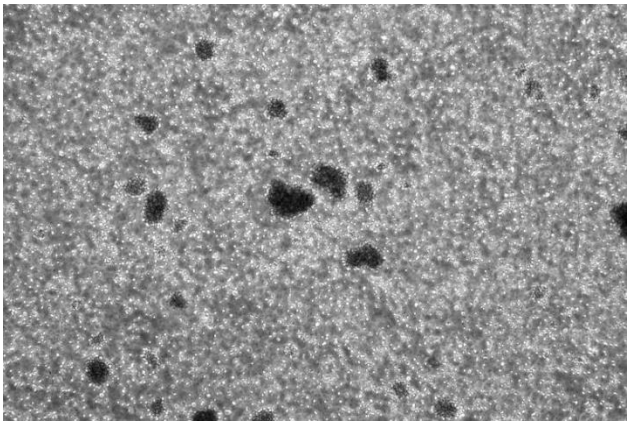
Project Description: Generation of NF-κB (Luc) for NK-92MI#NF-κB (Luc)#Poolcells with Lentivirus.

Generation of the over-expression cell lines:

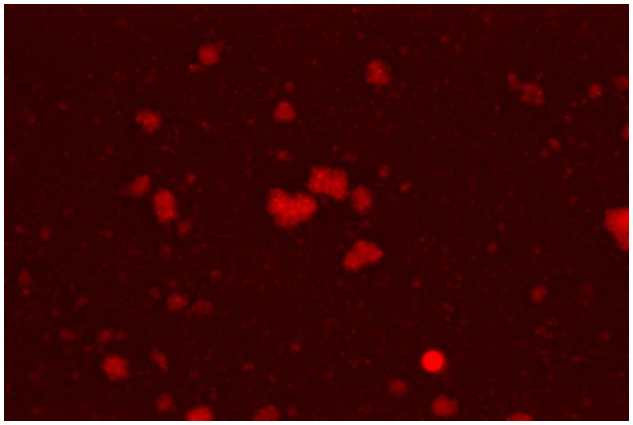
NK-92MI cells will be transduced with lentivirus particles below:

pLV[Exp]-NFkB-Luciferase-EF1A>mCherry:T2A:Puro

The morphology of the cell lines is shown below (40X magnification):



NK-92MI#NF-κB (Luc)#Poolcells-40x-W



NK-92MI#NF-κB (Luc)#Poolcells-40x-F(199ms)

Validation of the lentivirus insertion and GOI expression

RT-qPCR

RT-qPCR was performed on total RNA extracted from the three samples:

NK-92MI#NF-κB (Luc)#Poolcells, wildtype NK-92MI cells and ddH_2O.

Primer information

Gene	Primer	Primer Sequence (5'->3')	Length (bp)
NF-κB (Luc) (gene of interest)	F	TCACCGACGCACATATCGAG	156
	R	CCAACACGGGCATGAAGAAC	

Result

Group	NF-κB (Luc)	mGAPDH	Δ CT
NK-92MI#NF-κB (Luc)	21.26	16.08	5.18
NK-92MI	Undetectable	16.68	N/A
H_2O	Undetectable	Undetectable	N/A

Conclusion

The RT-qPCR results show that NF-κB (Luc) mRNA is efficiently expressed in NK-92MI#NF-κB (Luc)#Poolcells

8. Spot Inventory List

Note: Subject to actual inventory availability provided by the Sales Department.

Host Cell	Reporter Gene	Target Gene	Cat.No
SW780	Luciferase	/	ABC-RC214H
NCI N87	Luciferase	/	ABC-RC681H
Raji	Luciferase	/	ABC-RC206H
HepG2	Luciferase	/	ABC-RC176H
Caco-2	EGFP	/	ABC-RC163H
C2C12	EGFP	/	ABC-RC012Z
4T1	EGFP	/	ABC-RC002Z
MM.1S	Luciferase & GFP	/	ABC-RC661H
PLC/PRF/5	Luciferase & GFP	/	ABC-RC696H
NCI-H1975	Luciferase & GFP	/	ABC-RC671H
B16	Luciferase	OVA	ABC-RC578H
4T1	Luciferase	OVA	ABC-RC559H
HEK293	Luciferase	Mouse GDF15	ABC-RC115F
HEK293	Luciferase	Human GDF15	ABC-RC114F
HEK293	Luciferase	Human IL-5	ABC-RC126F
Our services are more than that...			

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