



## pLIVE™, pLIVE™-lacZ, and pLIVE™-SEAP Vectors Product # MIR 5420, 5520, 5620, 5320

### INTENDED USE

Mirus Bio Corporation has developed the pLIVE™ Vector (Liver In Vivo Expression), which is designed for high level, prolonged expression of transgenes in the mouse liver. This vector utilizes a chimeric promoter composed of the mouse alpha fetoprotein enhancer II and the minimal mouse albumin promoter. Two introns have been engineered into the expression plasmid so that they will be present in the primary transcript produced from the chimeric promoter, in turn increasing expression of the delivered transgene. Downstream of the first intron is a multiple cloning site (MCS) with eight unique restriction sites allowing for simple insertion of the gene of interest. Together the chimeric promoter and two introns are capable of promoting high level transgene expression in the liver for extended lengths of time compared to classic promoters such as the CMV immediate early promoter.

Two reporter vectors derived from pLIVE™, pLIVE™-lacZ and pLIVE™-SEAP, were created for use as positive controls. Expression of the lacZ gene from pLIVE™-lacZ can be monitored in the liver using either classical X-gal staining of liver sections or quantitative β-galactosidase assays of liver lysates. Expression of the SEAP gene from pLIVE™-SEAP can be easily monitored using a quantitative assay of mouse serum. The high level, long term, liver-specific expression of transgenes from the pLIVE™ Vector, as well as the availability of the positive control pLIVE™-lacZ and pLIVE™-SEAP Reporter Vectors make the pLIVE™ Vectors the ideal choice for *in vivo* liver expression studies in mice.

### FREQUENTLY ASKED QUESTIONS

#### General Questions:

**Q1. What is the *E. coli* selectable marker on the pLIVE™ Vectors?**

All the pLIVE™ Vectors carry the kanamycin resistance gene, and *E. coli* cells harboring these plasmids should be grown in media containing 30 µg/ml of kanamycin.

**Q2. Are the pLIVE™ Vectors high or low copy number plasmids?**

The plasmid backbone in these vectors is derived from pUC, making them high copy number plasmids. Mirus Bio scientists routinely obtain 500-750 µg of plasmid DNA from only 100 ml of LB + kanamycin bacterial cultures.

**Q3. What are the stability and storage conditions for the pLIVE™ Vectors?**

The vectors are supplied in a modified TE solution composed of 10 mM Tris, pH 7.5 and 0.1 mM EDTA. Store the vectors at -20°C and thaw at room temperature before use. These vectors are stable for at least 1 year from date of purchase if stored properly.

**Q4. What is the best method for preparing pLIVE™ Vector DNA before delivery?**

Scientists at Mirus Bio routinely use the Marligen High Purity Plasmid Purification Kits (Cat # 11452-018 and 11451-010) to purify DNA before delivery to mice. The DNA prepared using these kits usually contains about 100 endotoxin units (EU) per mg of DNA, and it is recommended that the residual endotoxin be removed using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (MIR 5900).

**Q5. What level of endotoxin in the pLIVE™ Vector DNA is acceptable for *in vivo* delivery?**

Less than 30 Endotoxin Units/mg of DNA is acceptable. The presence of more endotoxin in the injected DNA could lead to an adverse reaction in the mice after delivery.

**Q6. How does Mirus Bio quantify the amount of endotoxin in a DNA prep?**

We use the QCL-1000® Chromogenic LAL Endpoint Assay Kit from Cambrex Corp.



### **Q7. Which method does Mirus Bio recommend for delivery to the mouse liver?**

Mirus Bio recommends using the hydrodynamic tail vein injection procedure for the delivery of naked nucleic acids to the mouse liver. Mirus Bio has a family of hydrodynamic delivery companion products including the *TransIT*<sup>®</sup>-QR and *TransIT*<sup>®</sup>-EE Hydrodynamic Delivery Solutions. For additional products and product information, see the Mirus Bio website at: <http://www.mirusbio.com/products/index.asp>

**NOTICE:** *In vivo* hydrodynamic delivery of non-viral nucleic acids is covered by patents and patent applications of Mirus Bio Corporation, including U.S. Patent 6,627,616; 6,379,966 and related filings worldwide. Purchase of this product does not provide a license to this delivery technology, which is required for all research and commercial uses by for-profit entities. To inquire about a license, please contact Mirus Bio Corporation.

### **Q8. Are the pLIVE<sup>™</sup> Vectors compatible with other delivery methods?**

Hydrodynamic tail vein injection is currently the most efficient non-viral method for the delivery of nucleic acids to the liver. However, any method that can successfully deliver DNA to the liver will result in expression from the pLIVE<sup>™</sup> Vectors.

### **Q9. Will the pLIVE<sup>™</sup> Vectors function in rats?**

The chimeric promoter in the pLIVE<sup>™</sup> Vectors efficiently transcribes the transgene when delivered to the rat liver. If delivering the pLIVE<sup>™</sup> Vectors to the rat liver by hydrodynamic tail vein injection, we recommend increasing the amount of pLIVE<sup>™</sup> Vector DNA to 100-500 µg per injection.

### **Q10. Can the pLIVE<sup>™</sup> Vectors be used in co-delivery applications for RNAi experiments?**

Yes. These vectors can be co-delivered with siRNA to achieve RNAi-mediated gene knockdown of the target gene expressed from the pLIVE<sup>™</sup> Vector.

### **Q11. What elements are present in the pLIVE<sup>™</sup> Vector chimeric promoter?**

The chimeric promoter in the pLIVE<sup>™</sup> Vectors contains two important elements necessary for long-term, stable expression of transgenes in the liver. The first element is the mouse minimal albumin promoter, and the second element is the mouse alpha fetoprotein II enhancer.

### **Transgene Expression Questions and Answers:**

### **Q12. How do expression levels compare between the pLIVE<sup>™</sup> Vectors and vectors utilizing the cytomegalovirus immediate early promoter (CMV promoter) to drive transgene expression?**

We have compared the expression of the human placental secreted alkaline phosphatase gene (SEAP) from the pLIVE<sup>™</sup> Vector and a vector expressing the same reporter using the CMV promoter. Maximal expression from the CMV promoter is approximately 2.5 fold greater than the maximal expression achieved from the pLIVE<sup>™</sup>-SEAP Vector. However, expression from the CMV promoter rapidly decreases after delivery, and SEAP activity is negligible 10 days post-delivery. SEAP expression from the pLIVE<sup>™</sup> Vector increases from day 1 to day 4 post-injection and maintains that expression level for at least 8 months post-delivery. See the Mirus Bio website for additional experimental details: <http://www.mirusbio.com/products/transit/plive/index.asp>

### **Q13. How long will expression from the pLIVE<sup>™</sup> Vectors continue after delivery to the liver?**

Currently we have SEAP expression data out to 8 months post-injection in mice. The SEAP levels in these mice are not decreasing, which suggests that expression may continue for many more months.

### **Q14. What is the key factor that affects the duration of transgene expression from the pLIVE<sup>™</sup> Vector?**

The key factor affecting duration of transgene expression is the immune response by the mouse. If the protein expressed from the delivered transgene is immunogenic in the mouse, then the immune system will begin to destroy the liver cells expressing the transgene. An immune response to the transgene product will normally be evident by a rapid decrease in transgene expression 10-14 days post-injection.

**Q15. How can the duration of transgene expression be increased?**

The strain of mice used can have a dramatic effect on the duration of expression. In general, we have observed longer expression from the pLIVE™ Vectors when using C57Bl/6 mice compared to ICR mice. In addition, the more similar the transgene product is to its mouse counterpart, the longer the predicted duration of expression. Finally, if a mouse homolog exists for the transgene of interest, expressing that mouse homolog in place of the current transgene should prevent an immune response from occurring, thus enabling maximal long-term expression.

**Q16. How much vector DNA should be delivered to each mouse?**

When using hydrodynamic injections to deliver the pLIVE™ Vector DNA to the mouse liver, 10-50 µg of injected vector DNA is generally sufficient to obtain good transgene expression. However, the amount of DNA injected for a given experiment should be tested empirically to obtain the desired level of transgene expression. If using other delivery techniques, the amount of DNA will again have to be determined empirically. For hydrodynamic tail vein delivery to rats, inject 100-500 µg of vector DNA.

**Q17. Can two pLIVE™ Vectors expressing different transgenes be delivered simultaneously to the mouse liver?**

Yes. However, we have seen decreases in the level of expression of both transgenes over time.

**Q18. Will the presence of an intron downstream of the pLIVE™ MCS induce nonsense mediated decay (NMD)<sup>1,2</sup> of the transgene transcript?**

Possibly. In general, it is believed that if the stop codon of an open reading frame is more than 50 bases upstream of the last exon-exon junction, then this transcript will be degraded by the cell, a process known as nonsense mediated decay.<sup>1,2</sup> If a transgene open reading frame is cloned into the pLIVE™ Vector MCS such that the stop codon is adjacent to the *Sac* I, *Sac* II or *Xho* I sites, then the stop codon will be less than 50 bases upstream of the last exon-exon junction and NMD should be avoided. However, if a transgene open reading frame is cloned into the pLIVE™ Vector MCS such that the stop codon is adjacent to the *Nhe* I, *Asc* I, *Sal* I, *Sma* I or *Bam*H I sites, then the stop codon will be more than 50 bases upstream of the last exon-exon junction and NMD could be induced. We have cloned the firefly luciferase open reading frame into the pLIVE™ Vector such that the stop codon is adjacent to the *Nhe* I, *Sal* I, *Bam*H I, or *Xho* I sites. Maximal expression was seen when the stop codon was adjacent to the *Xho* I site, and was approximately 2-3 times greater than the expression observed from the other clones, suggesting that NMD can occur. However, luciferase expression from the *Nhe* I clone was still high and easily detected.

**Q19. Is the sequence surrounding the transgene start codon important for optimal translation?**

Yes. The context surrounding the ATG start codon of the transgene open reading frame can have a dramatic effect on the efficiency of translation.<sup>3,4</sup> Use the following sequence around the ATG (underlined) to promote optimal translation: (G/A)NNATGG.<sup>3,4</sup> If a G at the first nucleotide position after the ATG changes the amino acid encoded by that codon and that change will not be tolerated, then use the wildtype base at this position to avoid mutation of the transgene protein.

**pLIVE™-lacZ and pLIVE™-SEAP Reporter Vector Questions:**

**Q20. How can the expression of the lacZ reporter gene be assessed after delivery?**

After delivery of the pLIVE™-lacZ Vector, the livers can either be harvested, sectioned and stained with X-gal to stain the lacZ expressing cells blue, or the livers can be homogenized and the lysates can be assayed for β-galactosidase activity using a quantitative assay. For staining of the livers with X-gal, Mirus Bio recommends the Beta-galactosidase Staining Kit (MIR 2600). For the quantitative assay of liver lysates, Mirus Bio recommends using the chemiluminescent Galacto-Light™ Kit from Applied Biosystems.

**Q21. How should the liver lysates be treated when using the Galacto-Light™ Kit (Applied Biosystems) to quantify lacZ expression?**

After harvesting the livers, we normally lyse each liver in approximately 4 ml of lysis buffer (not supplied), and then dilute 100-500 fold before assaying according to the kit's protocol. Using samples that are undiluted will lead to a severe under-representation of the level of β-galactosidase activity in the liver, likely due to the inhibition of β-gal enzyme activity.



### Q22. What is SEAP?

SEAP stands for Secreted Alkaline Phosphatase, and is a truncated form of the human placental alkaline phosphatase gene. It is often used as a reporter for gene expression because the SEAP protein is secreted from the cells making harvest and assay of SEAP activity simple and rapid.<sup>5</sup> Using SEAP as a reporter plasmid does not require sacrificing the animal and allows for long term multi-sampling of the same animal.

### Q23. How can the expression of the SEAP reporter gene be assessed after delivery?

After delivery of the pLIVE™-SEAP Vector, the serum is harvested and assayed for SEAP activity using a quantitative assay such as the Phospha-Light™ Kit from Applied Biosystems.

### Q24. How should the serum samples be treated when assaying for SEAP activity?

Depending on the delivery efficiency and expression level of the pLIVE™-SEAP Vector, we generally dilute the serum samples 100-500 fold before assaying using the Phospha-Light™ Kit (Applied Biosystems). This level of dilution normally produces SEAP activities that are within the useful range of the standard curve generated using purified SEAP protein.

### Troubleshooting Questions:

### Q25. I am not seeing any expression of either the *lacZ* or SEAP reporter genes after delivery. What could be causing this problem?

#### Poor delivery of the pLIVE™-*lacZ* and pLIVE™-SEAP Vectors

It is possible that the delivery method being used is not effectively delivering the vectors to the liver. If using hydrodynamic tail vein injections, be certain to inject a nucleic acid solution equivalent to 1/10 the volume of the mouse with 10 µg of vector DNA. Also be certain to administer the entire solution into the tail vein in one smooth injection over 4-7 seconds. Use young, approximately 20 g mice for hydrodynamic tail vein injections. Larger mice generally produce lower and less reproducible levels of transgene expression compared to 20 g mice. For additional assistance with hydrodynamic delivery, see our *TransIT*®-QR Hydrodynamic Delivery Solution protocol (ML041), email our technical support team at techsupport@mirusbio.com, or call 1.888.530.0801.

### Q26. I am not seeing any expression of my transgene after delivery. What could be causing this problem?

#### Poor delivery to the pLIVE™ Transgene Expression Vector

See the answer to Q24 above.

#### Suboptimal sequence surrounding the ATG start codon of the transgene.

See Q14 and design the sequence surrounding the ATG start codon (underlined) to have the following sequence: (G/A)NNATGG.<sup>3,4</sup> This sequence should promote optimal translation efficiency.

#### Suboptimal location of the transgene stop codon in the pLIVE™ Vector MCS (see question 17)

If the stop codon of the transgene open reading frame is more than 50 bases upstream of the 5' end of intron 2 (base 988), nonsense mediated decay could be induced in the cell, leading to a decreased level of the transgene mRNA in the cell.<sup>1,2</sup> We have seen a 2-3 fold decrease in the level of luciferase expression when the luciferase stop codon is more than 50 bp upstream of the start of intron 2. However, we have not observed a total absence of transgene expression when the stop codon is more than 50 bases upstream of intron 2.

#### The protein product expressed from the transgene induces an immune response

If the transgene protein is immunogenic in mice, it will induce an immune response that will ultimately lead to clearance of the liver cells expressing the transgene. An immune response will usually take 10-14 days to develop and clear the cells. Harvest the livers and assay for transgene expression at an earlier timepoint. Alternatively, use C57Bl/6 mice which usually develop less robust immune responses compared to strains such as ICR.

For specific questions or concerns, please contact our Technical Support Team at 888.530.0801 or techsupport@mirusbio.com.



### REFERENCES

1. Beyers, P.H. (2002) *J. Clin. Invest.* 109:3-6.
2. Baker, K.E. and R. Parker (2004) *Curr. Opin. Cell Biol.* 16:293-299.
3. Kozak, M. (1987) *Nuc. Acids Res.* 15:8125-8148.
4. Kozak, M. (1991) *J. Cell Biol.* 115:887-903.
5. Ausubel, F.M. *et al.* (2005) *Current Protocols in Molecular Biology* John Wiley & Sons, Inc. Hoboken, New Jersey.

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\*Patent pending.

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