

REPORTER SYSTEMS

BioLux<sup>®</sup>  
*Cypridina* Luciferase Assay Kit

Instruction Manual

NEB #E3309S/L  
100/1,000 assays  
Version 1.1 3/13



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Japanese Patent Nos. 4,761,150 and 4,484,429

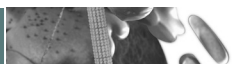
Japanese Appln. Serial No.: 2006-280827; 2007-536587 and 2009-257631

EPO Appln. Serial No.: 06 810 525.3

Chinese Appln. Serial No.: 200680035410.3

For use of the Biolux Cypridina Luciferase Assay Kit, or associated assay reagents, in human diagnosis and measurement in relation to human health, contact [busdev@neb.com](mailto:busdev@neb.com).

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## Kit Includes:

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|   | #E3309S            | #E3309L   |
|---|--------------------|-----------|
| BioLux <i>Cypridina</i> Luciferase Assay Buffer (1X) .....              | 5 ml               | 2 x 25 ml |
| BioLux <i>Cypridina</i> Luciferase Substrate Solvent <sup>†</sup> ..... | 0.5 ml             | 0.5 ml    |
| BioLux <i>Cypridina</i> Luciferase Substrate .....                      | lyophilized powder |           |

*(Kit components are not sold separately)*

<sup>†</sup> *The BioLux *Cypridina* Luciferase Substrate Solvent must be completely thawed and diluted with absolute ethanol (not included) before using it to make the 100X substrate solution (please refer to the section of Reconstitution of BioLux *Cypridina* Luciferase Substrate).*

## Storage Information:

The BioLux *Cypridina* Luciferase Assay Kit should be stored at  $-20^{\circ}\text{C}$ .

### **IMPORTANT:**

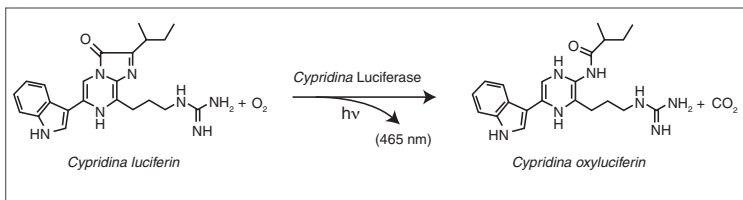
BioLux *Cypridina* Luciferase Assay Buffer (1X) and Substrate (lyophilized powder) must always be protected from light.

BioLux *Cypridina* Luciferase Substrate is supplied in lyophilized form to ensure long shelf life and maximum stability.

## Description:

The BioLux® *Cypridina* Luciferase Assay Kit contains the reagents necessary for assaying *Cypridina* Luciferase activity. *Cypridina* is a new secreted luciferase reporter from the ostracod *Cypridina noctiluca*. This luciferase does not require ATP and catalyzes the oxidation of its luciferin substrate in a photochemical reaction (1,2) (Figure 1). The substrate for *Cypridina* is different than coelenterazine, which is the common substrate of other marine luciferases including *Renilla* and *Gaussia*.

Figure 1: The photochemical reaction catalyzed by *Cypridina* Luciferase.



## Properties of *Cypridina* Luciferase:

*Cypridina* Luciferase is secreted from cells by virtue of its natural signal peptide and its luminescence can be measured from the supernatant of transfected cells. Therefore, cell lysis is not necessary (Figure 2).

Secreted CLuc is a very stable protein. Because of this property, the activity measured from the supernatant reflects the amount of protein accumulated up to the time of sampling. Multiple samples can therefore be obtained from the same transfected cells (Figure 2).

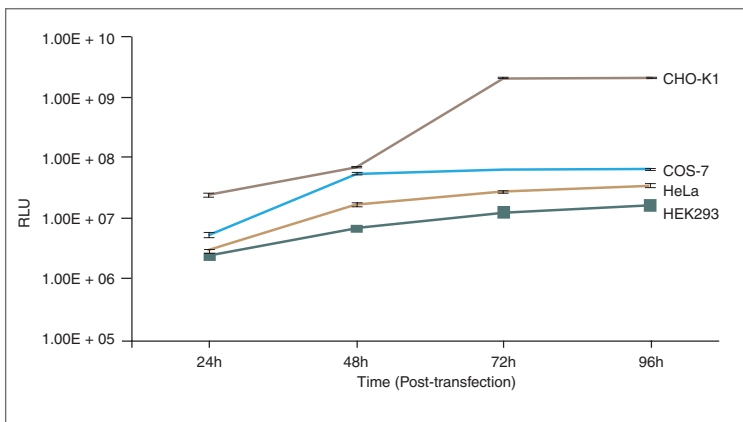
The CLuc assay is very sensitive, allowing detection of very small amounts of *Cypridina* Luciferase activity (Figure 3).

Secreted CLuc is thermally stable at 55°C (Figure 4A), which is the typical inactivation temperature of most viruses.

Secreted CLuc remains active in the presence of  $\beta$ -mercaptoethanol, which is commonly present in the complete culture medium of mouse ES cells (Figure 4B).

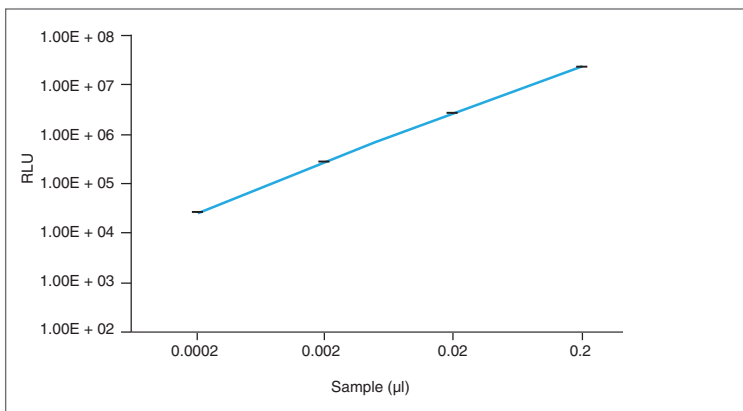
Although the light reaction catalyzed by *Cypridina* has an initial emission half-life of approximately 5 minutes, light production continues to decay slowly, and is readily detectable 25 minutes after substrate addition (Figure 5).

Figure 2: Activity of secreted CLuc at different time points.



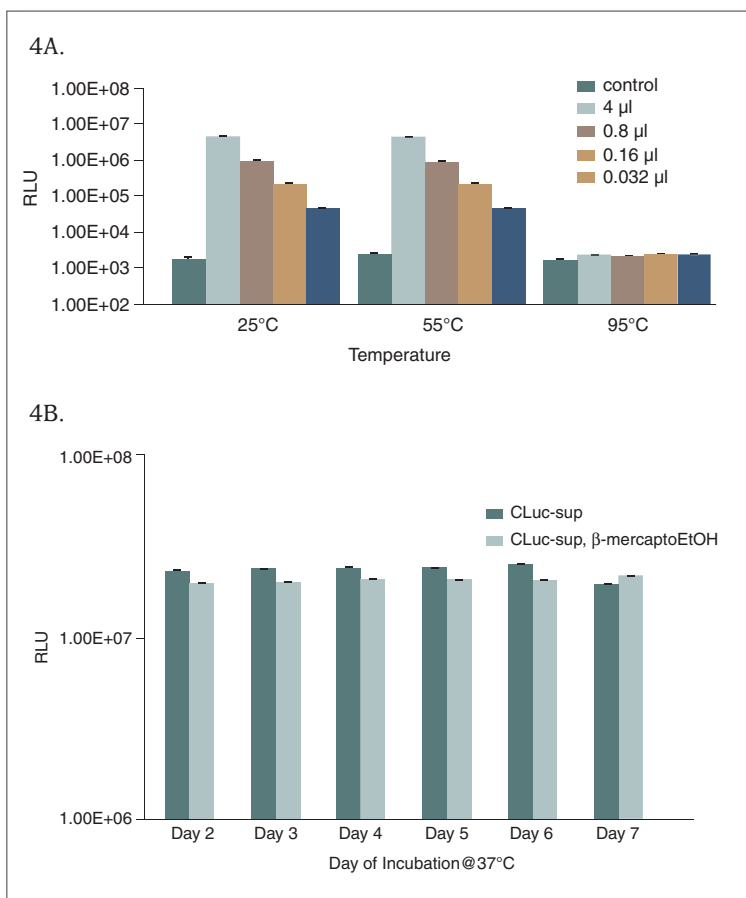
Supernatants of transfected cells were collected and medium was replaced each day for 4 days. These supernatants were stored at  $-20^{\circ}\text{C}$  until the last samples were obtained. Relative Light Units, RLU.

Figure 3: Sensitivity of *Cypridina* Luciferase assay.



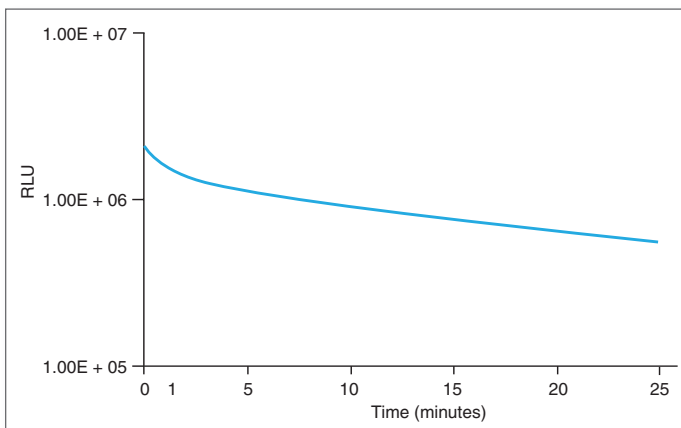
The CLuc activity was assayed from 10-fold serial dilutions of the supernatant from a stable CLuc-expressing cell line.

Figure 4: Stability of *Cypridina* Luciferase.



(A) Supernatant obtained from stable CLuc-expressing cells was incubated at 55°C or 95°C for 30 minutes and allowed to cool to room temperature (25°C). Five-fold serial dilutions of supernatant were assayed for CLuc activity. The control is the supernatant of the parental cells from which the stable cell line was created. (B) Supernatants from stable CLuc-expressing cells, grown in medium with or without  $\beta$ -mercaptoethanol, were incubated at 37°C and assayed each day for 7 days.

Figure 5: Kinetics of light emission.



The *Cypridina* Luciferase kinetic assay was performed with the supernatant from HeLa cells transiently transfected with a CLuc-expressing vector. The assay solution was prepared and incubated at room temperature for 30 minutes before use.

## Reconstitution of BioLux *Cypridina* Luciferase Substrate:

### IMPORTANT:

Lyophilized substrate can be stored at  $-20^{\circ}\text{C}$  for up to 2 years without loss of activity. Reconstituted substrate should be prepared only if you plan to perform the assays in the near future.

Aliquots of reconstituted substrate can be stored at  $-80^{\circ}\text{C}$  for up to 2 months.

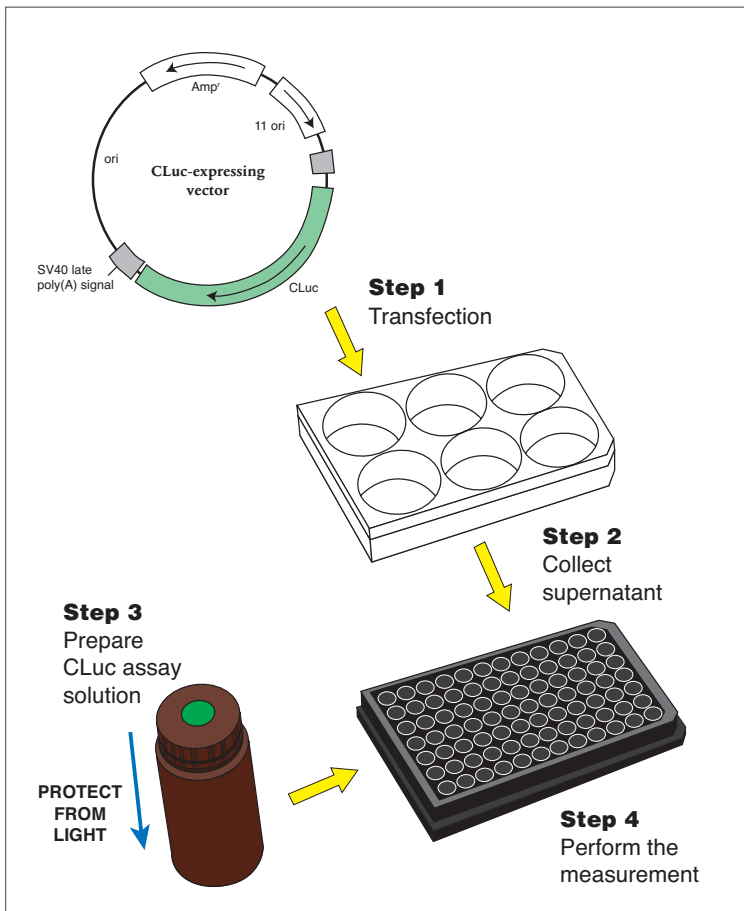
### Preparation of Reconstituted Substrate (100X solution):

1. Briefly centrifuge the thawed BioLux *Cypridina* Substrate Solvent vial at 12,000 rpm for 30 seconds.
2. Add 0.5 ml of absolute ethanol (not included) to the BioLux *Cypridina* Substrate Solvent and mix well.
3. Carefully lift the rubber cap of the lyophilized BioLux *Cypridina* Substrate vial and add 60  $\mu\text{l}$  of the ethanol-solvent mixture (for small kit) or 600  $\mu\text{l}$  of the ethanol-solvent mixture (for large kit) to the lyophilized BioLux *Cypridina* Luciferase Substrate.
4. Gently mix to dissolve the substrate (do not vortex, do not create bubbles by pipeting) and incubate at  $4^{\circ}\text{C}$  for 10–15 minutes (protect from light).
5. Gently mix again and incubate at  $4^{\circ}\text{C}$  for an additional 10–15 minutes (protect from light).

Note: Be sure to dissolve the residual lyophilized powder on the rubber cap.

6. Aliquot the reconstituted substrate (100X solution) and store at  $-80^{\circ}\text{C}$ .

Figure 6: *Cypridina* Luciferase Assays



## CLuc Assay Kit Protocols

### Protocol I (Luminometers without injectors):

1. Thaw BioLux *Cypridina* Luciferase Assay Buffer (1X) completely to room temperature (protect from light) and mix well before use.
2. Prepare the CLuc assay solution (e.g. for 100 samples add 50  $\mu$ l of the reconstituted substrate (100X solution) to 5 ml of BioLux *Cypridina* Luciferase Assay Buffer).
3. Mix well by inverting the tube several times (do not vortex).



4. Incubate at room temperature for 30 minutes (protect from light).
5. Set the luminometer for 2–10 seconds of integration.
6. Pipet samples \*(5–20  $\mu$ l per well) into a 96-well white (opaque), black plate or cuvette.
7. Add the CLuc assay solution (50  $\mu$ l) to a sample (i.e. add the assay solution to only one sample at a time) and promptly proceed with the measurement.
8. Repeat Step 7 for all samples.

#### Protocol II (Injector-equipped luminometers):

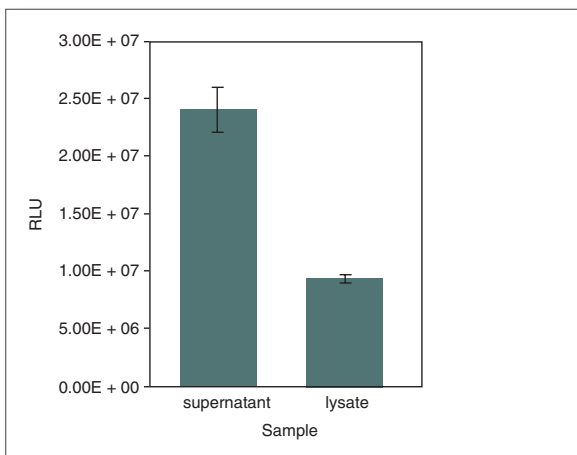
1. Thaw BioLux *Cypridina* Luciferase Assay Buffer (1X) completely to room temperature (protect from light) and mix well before use.
2. Prepare the CLuc assay solution (e.g. for 100 samples add 50  $\mu$ l of the reconstituted substrate (100X solution) to 5 ml of BioLux *Cypridina* Luciferase Assay Buffer).

Note: Be sure to prepare enough CLuc assay solution as needed for all samples as well as for priming the injector as suggested by the manufacturer.

3. Mix well by inverting the tube several times (do not vortex).
4. Incubate at room temperature for 30 minutes (protect from light).
5. Set the luminometer with the following parameters: 50  $\mu$ l injection, 1–2 seconds of delay and 2–10 seconds of integration.
6. Pipet samples \*(5–20  $\mu$ l per well) into a 96-well white (opaque) or black plate.
7. Prime the injector with the CLuc assay solution and proceed with the measurement.

\* Approximately 90% of *Cypridina* Luciferase is secreted into the culture medium after transfection and thus, the CLuc activity is typically assayed in the supernatant (i.e. culture medium of CLuc-transfected cells). However, as long as the cells are alive, ~10% of CLuc is present inside the cell and therefore, the CLuc activity can also be assayed in the cell lysate (Figure 7). We recommend that the cell lysates be prepared by using Luciferase Cell Lysis Buffer (#B3321), since this lysis buffer is designed to be compatible with *Cypridina*, *Gaussia*, *Renilla*, Firefly Luciferase and  $\beta$ -gal activity assays.

Figure 7: Activity of *Cypridina* Luciferase in the supernatant and lysate from a stable CLuc-expressing cell line.



The CLuc activity was measured from 20  $\mu$ l of supernatant (from 500  $\mu$ l total volume) and from 20  $\mu$ l of cell lysate (100  $\mu$ l total lysate volume) of a stable CLuc expressing cell line. .

## Usage Notes:

Store the BioLux *Cypridina* Luciferase Assay Buffer (1X) at  $-20^{\circ}\text{C}$  for up to 2 years.

The BioLux *Cypridina* Luciferase Assay Buffer (1X), the reconstituted CLuc substrate (100X solution) and the CLuc assay solution must be protected from light.

The CLuc assay solution should be freshly prepared and incubated at room temperature for 30 minutes (protect from light) before use.

The linear range of the luminometer must be established. This can be easily done by assaying serial dilutions of a CLuc containing sample (Figure 3), such as the culture medium of CLuc-expressing cells. In addition, the assay solution itself as well as the conditioned medium (culture medium from untransfected cells) should be included to establish the background in the assay.

If excess activity for the instrument range is found, the sample should be diluted in either PBS or 10% serum-containing medium. The integration time can also be reduced, e.g. 2 seconds instead of 5 seconds.

The presence of serum, i.e. culture medium, typically increases the background signal in the assay. For example, the CLuc assay solution alone shows  $10^1$ – $10^2$  RLU; the CLuc assay solution in 10% FBS-containing medium results in  $10^2$ – $10^4$  RLU, depending on the type of medium used.

The recommended integration time for the luminescence measurement is 2–10 seconds. It is important to keep the integration time constant, in order to obtain consistent results.

## Frequently Asked Questions:

### ***Can I save the unused portion of the CLuc assay solution?***

Ideally the CLuc assay solution is prepared immediately before performing the assays. However, the unused portion of the assay solution can be stored at  $-20^{\circ}\text{C}$  for up to 2 days. It must be completely thawed to room temperature (protected from light) and mixed well (do not vortex) before use. Using the previously frozen assay solution will result in approximately a 20% decrease in CLuc activity. The unused portion of the CLuc assay solution should be discarded after one round of freeze-and-thaw.

### ***Does the activity of Cypridina Luciferase interfere with that of other luciferases such as Gaussia, Renilla & Firefly?***

No. *Cypridina* Luciferase catalyzes the light production of a substrate that is different than those used by *Gaussia*, *Renilla* or Firefly luciferases. The secreted CLuc is typically assayed in the supernatant of CLuc-expressing cells; however, the CLuc activity can also be assayed in the cell lysate. If the cell lysate contains *Gaussia*, *Renilla* or Firefly luciferase in addition to *Cypridina* Luciferase, one can assay each luciferase independently without cross-reactivity.

### ***What other reporter can I use along with Cypridina?***

*Cypridina* can be used in combination with *Gaussia*, *Renilla* and Firefly luciferases and *lacZ* reporter in a co-transfection. *Cypridina* and *Gaussia* are both secreted luciferases, producing high bioluminescent signal intensity. Thus, *Cypridina* and *Gaussia* are an ideal reporter pair for co-transfecting mammalian cells (3). For more information on *Gaussia* Luciferase products, please go to the website, ([www.neb.com](http://www.neb.com)).

## References:

1. Nakajima, Y. et al. (2004) *Biosci. Biotechnol. Biochem* 63, 565–570.
2. Yamagishi, K., Enomoto, T. and Ohmiya, Y. (2006) *Anal. Biochemistry*, 354, 15–21.
3. Wu, C., Suzuki-Ogoh, C. and Ohmiya, Y. (2007) *Biotechniques*, 42, 290–292.

## Ordering Information

| Product   | NEB #    | Size             |
|---|----------|------------------|
| BioLux <i>Cypridina</i> Luciferase Assay Kit    | E3309S/L | 100/1,000 assays |
| <b>Companion Products</b>                       |          |                  |
| pSV40-CLuc Control Plasmid                      | N0318S   | 20 µg            |
| pCLuc-Basic 2 Vector                            | N0317S   | 20 µg            |
| pCMV-CLuc 2 Control Plasmid                     | N0321S   | 20 µg            |
| pTK-CLuc Vector                                 | N0322S   | 20 µg            |
| pCLuc Mini-TK 2 Vector                          | N0324S   | 20 µg            |
| BioLux <i>Cypridina</i> Luciferase Starter Kit  | E3314S/L | 100/1,000 assays |
| pSV40-GLuc Control Plasmid                      | N0323S   | 20 µg            |
| pGLuc-Basic 2 Vector                            | N8082S   | 20 µg            |
| pCMV-GLuc 2 Control Plasmid                     | N8081S   | 20 µg            |
| pTK-GLuc Vector                                 | N8084S   | 20 µg            |
| pGLuc Mini-TK 2 Vector                          | N8086S   | 20 µg            |
| BioLux <i>Gaussia</i> Luciferase Assay Kit      | E3300S/L | 100/1,000 assays |
| BioLux <i>Gaussia</i> Luciferase Flex Assay Kit | E3308S/L | 100/1,000 assays |
| Anti-GLuc Antibody                              | E8023S   | 0.2 ml           |
| Luciferase Cell Lysis Buffer                    | B3321S   | 25 ml            |









## USA

New England Biolabs, Inc.  
240 County Road  
Ipswich, MA 01938-2723  
Telephone: (978) 927-5054  
Toll Free: (USA Orders) 1-800-632-5227  
Toll Free: (USA Tech) 1-800-632-7799  
Fax: (978) 921-1350  
e-mail: [info@neb.com](mailto:info@neb.com)  
[www.neb.com](http://www.neb.com)

## CANADA

New England Biolabs, Ltd.  
Telephone: (905) 665-4632  
Toll Free: 1-800-387-1095  
Fax: (905) 665-4635  
Fax Toll Free: 1-800-563-3789  
e-mail: [info.ca@neb.com](mailto:info.ca@neb.com)  
[www.neb.ca](http://www.neb.ca)

## CHINA

New England Biolabs (Beijing), Ltd.  
Telephone: 010-82378265/82378266  
Fax: 010-82378262  
e-mail: [info@neb-china.com](mailto:info@neb-china.com)  
[www.neb-china.com](http://www.neb-china.com)

## FRANCE

New England Biolabs France  
Free Call: 0800-100-632  
Free Fax: 0800-100-610  
e-mail: [info.fr@neb.com](mailto:info.fr@neb.com)  
[www.neb-online.fr](http://www.neb-online.fr)

## GERMANY & AUSTRIA

New England Biolabs GmbH  
Telephone: +49/(0)69/305 23140  
Free Call: 0800/246 5227 (Germany)  
Free Call: 00800/246 52277 (Austria)  
Fax: +49/(0)69/305 23149  
Free Fax: 0800/246 5229 (Germany)  
e-mail: [info.de@neb.com](mailto:info.de@neb.com)  
[www.neb-online.de](http://www.neb-online.de)

## JAPAN

New England Biolabs Japan, Inc.  
Telephone: +81 (0)3 5669 6191  
Fax: +81 (0)3 5669 6192  
e-mail: [info.jp@neb.com](mailto:info.jp@neb.com)  
[www.nebj.jp](http://www.nebj.jp)

## SINGAPORE

New England Biolabs Pte. Ltd.  
Telephone: +65 638 59623  
Fax: +65 638 59617  
e-mail: [sales.sg@neb.com](mailto:sales.sg@neb.com)  
[www.neb.sg](http://www.neb.sg)

## UNITED KINGDOM

New England Biolabs (UK) Ltd.  
Telephone: (01462) 420616  
Call Free: 0800 318486  
Fax: (01462) 421057  
Fax Free: 0800 435682  
e-mail: [info.uk@neb.com](mailto:info.uk@neb.com)  
[www.neb.uk.com](http://www.neb.uk.com)