Promega

NanoBiT® Technology

Monitor the Dynamics of Protein:Protein Interactions in Live Cells

NanoBiT® Technology

NanoLuc® Binary Technology (NanoBiT®) enables the set-up of real-time assays to monitor the dynamics of protein:protein interactions (PPIs) in live cells. The high sensitivity of NanoBiT® facilitates the measurement of PPIs even at low expression levels and can be applied to PPI modulator screens in 96-, 384- or 1534-well formats. Unlike related approaches based on split fluorescent proteins, NanoBiT® complementation is fully reversible and allows the measurement of both protein association and dissociation over a broad dynamic range.

Principle

The NanoBiT® system is based on two small subunits, Large BiT (LgBiT) and Small BiT (SmBiT), of the very bright NanoLuc® luciferase [1]. The LgBiT and SmBiT subunits have been independently optimized for stability and minimal self-association [2] and are expressed as fusions to target proteins of interest. Interaction of the target proteins facilitates subunit complementation to reconstitute a bright, luminescent enzyme. PPI dynamics can be followed in real-time inside live cells using the Nano-Glo® Live Cell Assay System, a nonlytic detection reagent that contains an optimized cell-permeable substrate.

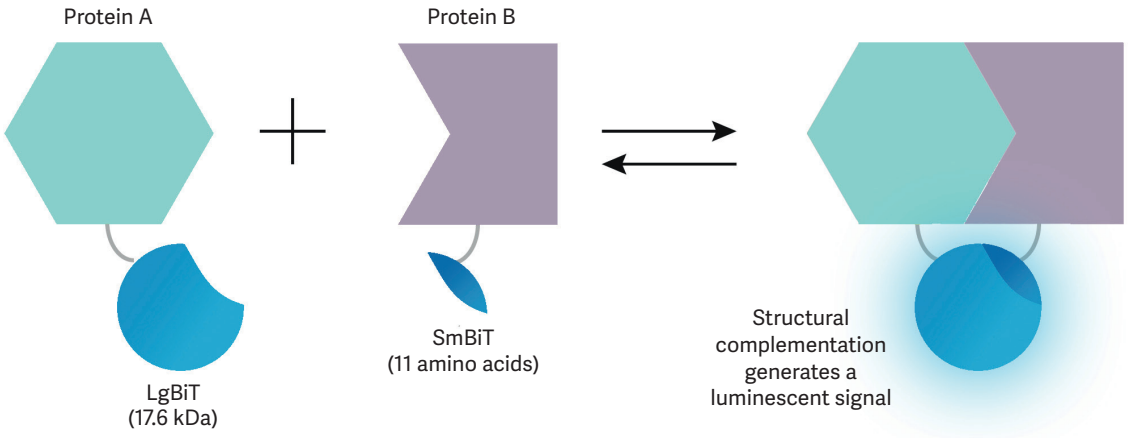


Figure 1: Structural complementation of the two optimized subunits upon protein A:protein B interaction. For a PPI of interest, interactors are expressed as fusion proteins to LgBiT and SmBiT, respectively. Interaction of fusion partners leads to structural complementation of LgBiT with SmBiT, generating a functional enzyme with a bright, luminescent signal.

References

[1] Hall, MP *et al.* (2012) Engineered luciferase reporter from a deep sea shrimp utilizing a novel imidazopyrazinone substrate. *ACS Chem Biol.* 2012 Nov 16.

[2] Dixon, A *et al.* (2015) NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. *ACS Chem Biol.* 2015 Nov 16.

NanoBiT® Features & Assay Workflow

Features

Physiological Expression Levels: Bright NanoLuc® allows the set-up of NanoBiT® assays with weak promoters for low expression levels.

High Sensitivity/Dynamic Range: Bright signal and reduced background improve sensitivity, signal:background ratio and dynamic range.

Highly Dynamic System: Reversible association of BiTs allows the study of rapidly associating or dissociating proteins in real-time.

Real-Time Measurement in Live Cells: Nonlytic assay reagent.

Small Tag Size: Minimize the risk of steric hindrance.

High-throughput Approved: 96-, 384-, 1536-well format.

Perform Simple Measurement: Bright luminescent output is ideal for any luminometer with no specific filter or injector requirements.

NanoBiT® Assay Workflow

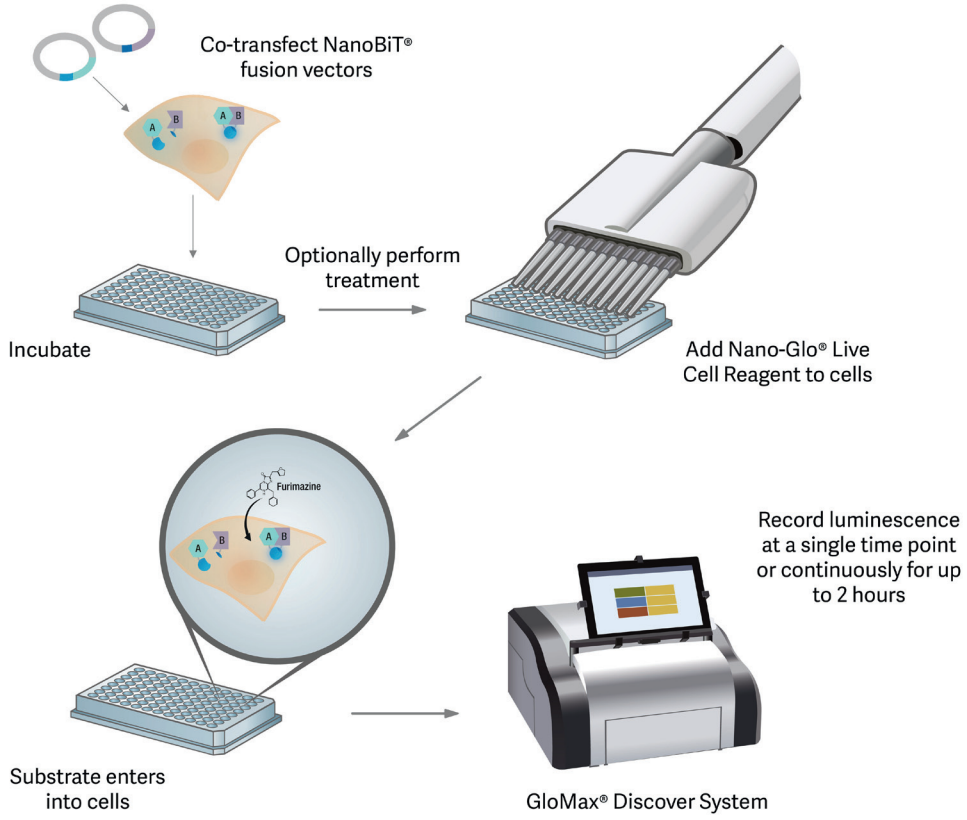


Figure 2: Overview of the NanoBiT® PPI Workflow. For a PPI of interest, the coding sequences of the interactors are cloned into LgBiT and SmBiT fusion vectors which are co-transfected into cells. Following expression of the fusion proteins, their interaction can be monitored in real-time inside live cells using the Nano-Glo® Live Cell Reagent. Luminescence is recorded with a plate reader such as the GloMax® Discover System at a single time point or continuously for up to 2 hours.

Applications of NanoBiT® in Live Cells

NanoBiT® enables the set up of protein:protein interaction assays to monitor the dynamics of protein association and dissociation in real-time. Areas of applications are:

- Real-time kinetic measurements of PPI dynamics
- PPI modulator screens in 96-, 384- or 1536-well formats
- Single-copy or single-cell measurements of PPI
- Cell-based sensors for e.g. GPCR activation
- Dose-response measurements
- Reporter cell lines

Examples of use

Real-time measurement of ligand-induced ß-arrestin 2 interaction with class A and class B GPCRs

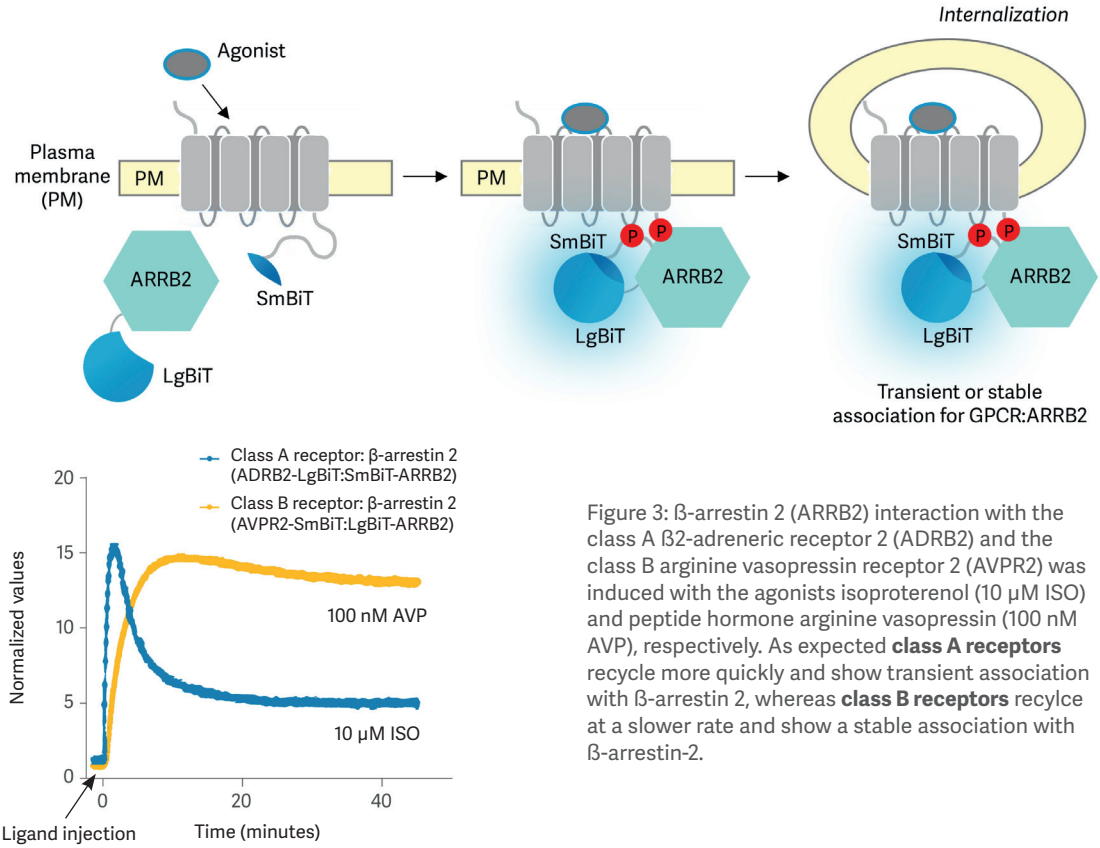


Figure 3: ß-arrestin 2 (ARRB2) interaction with the class A ß2-adreneric receptor 2 (ADRB2) and the class B arginine vasopressin receptor 2 (AVPR2) was induced with the agonists isoproterenol (10 µM ISO) and peptide hormone arginine vasopressin (100 nM AVP), respectively. As expected **class A receptors** recycle more quickly and show transient association with ß-arrestin 2, whereas **class B receptors** recylce at a slower rate and show a stable association with ß-arrestin-2.

Inhibition of p53:MDM2 interaction by Nutilin-3

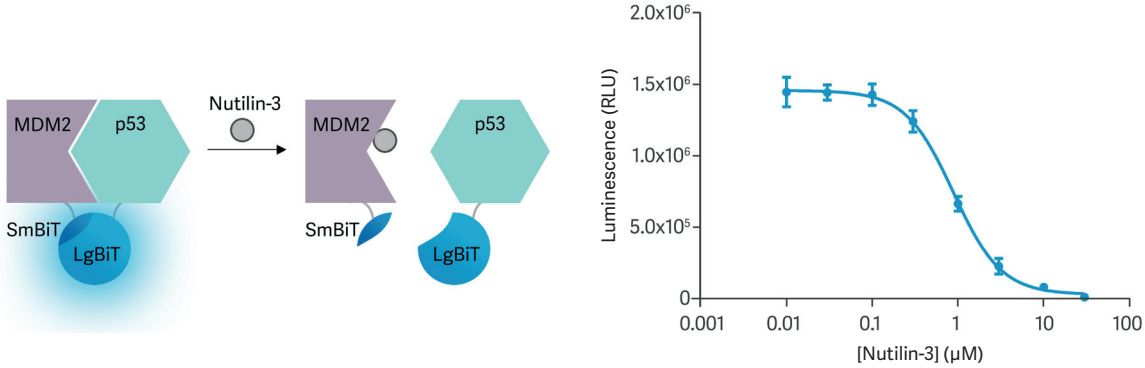
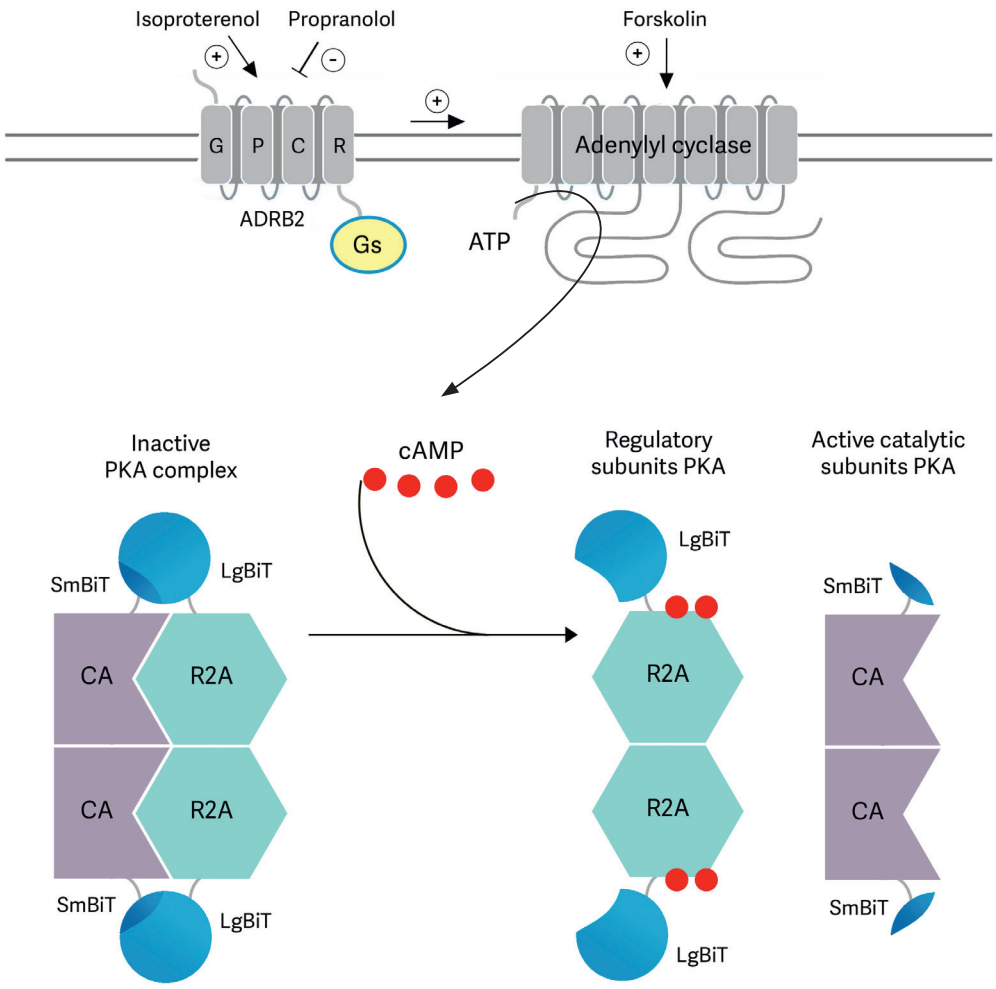


Figure 4: Dose-dependent inhibition of p53:MDM2 interaction by Nutilin-3. Performed in HEK293 cells after transient transfection of LgBiT-p53 and SmBiT-MDM2 fusion constructs.

Examples of use continued

Reversibility of NanoBiT® complementation as demonstrated by association and dissociation of the protein kinase A (PKA) complex



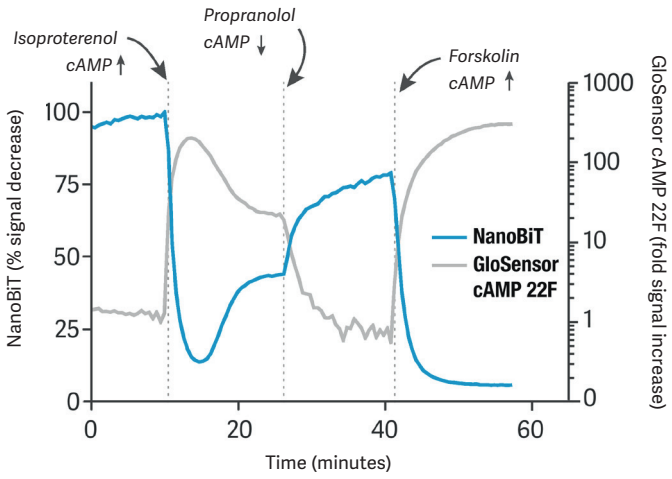
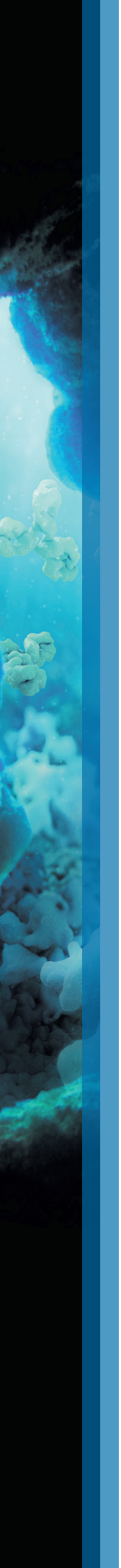


Figure 5: Real-time measurement of cAMP-dependent protein kinase A (PKA) complex association and dissociation using NanoBiT®. The activity of the protein kinase A complex is controlled by the regulatory PKA subunit (**R2A**) which dissociate from the catalytic subunits (**CA**) upon binding of **cAMP**. HEK293 cells were transfected with vector constructs expressing the catalytic subunit fused to SmBiT (SmBiT-PRKACA) and the regulatory subunit fused to the LgBiT (LgBiT-PRKAR2A). Intracellular levels of cAMP were modulated by the sequential addition of Isoproterenol (ADRB2 agonist), Propranolol (ADRB2 antagonist) and Forskolin (activator of adenylyl cyclase). The NanoBiT® PRKACA:PRKAR2A interaction was readily reversible showing rapid dissociation in response to increased cAMP and rapid association in response to decreased cAMP. Changes in intracellular cAMP levels were confirmed using the GloSensor™ cAMP assay in an independent experiment, which shows the expected inverse correlation to the NanoBiT® signal.



NanoBiT® PPI Starter Systems

The NanoBiT® PPI Starter Systems provide the vectors required to generate LgBiT and SmBiT protein fusions, a positive control pair (PRKACA:PRKAR2A) and a negative control vector. Starter systems also include the Nano-Glo® Live Cell Assay System, a single-addition, non-lytic detection reagent used for monitoring NanoBiT® luminescence in live cells. A rapamycin-inducible positive control (FKBP:FRB pair) is available separately.

Using the **NanoBiT® PPI MCS Starter System**, N- and C-terminal LgBiT and SmBiT fusions to proteins of interest are generated by using traditional cloning with a multiple cloning site (MCS). Expression is driven by HSV-TK promoter, providing constitutive, low-level expression in mammalian cells. The **NanoBiT® PPI Flexi® Starter System**, allows for the generation of BiT fusions using the Flexi® Vector Cloning System. The Flexi® Vector Cloning System is a directional cloning method based on the rare-cutting restriction enzymes, SgfI and PmeI, that provides a rapid, efficient and high-fidelity way to transfer protein-coding regions between Flexi® Vectors without the need to resequence.

The Find My Gene™ Human ORF clone collection (www.promega.com/findmygene) contains many ORF clones already in Flexi® format for a simple transfer in Flexi® vectors containing the LgBiT and SmBiT.

NanoBiT® PPI Starter Systems		Size	Cat.No.
NanoBiT® PPI MCS Starter System		1x each*	N2014
2 × 125 µl Nano-Glo® Live Cell Substrate 2 × 2.5 ml Nano-Glo® LCS Dilution Buffer			
20 µg pBiT1.1-C [TK/LgBiT] Vector 20 µg pBiT2.1-C [TK/SmBiT] Vector 20 µg pBiT1.1-N [TK/LgBiT] Vector 20 µg pBiT2.1-N [TK/SmBiT] Vector	*MCS cloning vectors for the generation of BiT fusion proteins*		
20 µg SmBiT-PRKACA Control Vector 20 µg LgBiT-PRKAR2A Control Vector	*Positive control PPI pair*		
20 µg NanoBiT® Negative Control Vector (HaloTag-SmBiT)			
NanoBiT® PPI Flexi® Starter System		1x each*	N2015
2 × 125 µl Nano-Glo® Live Cell Substrate 2 × 2.5 ml Nano-Glo® LCS Dilution Buffer			
20 µg pFC34K LgBiT TK-neo Flexi® Vector 20 µg pFC36K SmBiT TK-neo Flexi® Vector 20 µg pFN33K LgBiT TK-neo Flexi® Vector 20 µg pFN35K SmBiT TK-neo Flexi® Vector	*Flexi® cloning vectors for the generation of BiT fusion proteins*		
20 µg SmBiT-PRKACA Control Vector 20 µg LgBiT-PRKAR2A Control Vector	*Positive control PPI pair*		
20 µg NanoBiT® Negative Control Vector (HaloTag-SmBiT) 20 µg pF4A CMV Flexi® Vector (= Entry Vector)			

* Each system contains vectors for cloning and sufficient reagent for 200 assays in 96-well plates.

Available separately: inducible protein:protein interaction pair as additional positive control

NanoBiT® PPI Control Pair (FKBP, FRB)	N2016

Detection Reagent for NanoBiT® PPI: Nano-Glo® Live Cell Assay System

The Nano-Glo® Live Cell Assay System is a single-addition, non-lytic detection reagent used to measure NanoBiT® luminescence from live cells. The detection reagent contains the cell permeable substrate furimazine and a proprietary agent for reducing autoluminescence and decreasing assay background. The reagent is prepared by diluting the Nano-Glo® Live Cell Substrate with the Nano-Glo® LCS Dilution Buffer to make the Nano-Glo® Live Cell Reagent. Both, substrate and buffer solutions, are optimized to provide enhanced stability and reduce autoluminescence in the presence or absence of serum, increasing the sensitivity for detection of low levels of NanoBiT® luminescence.

Detection Reagent NanoBiT®	Size	Cat.No.
Nano-Glo® Live Cell Assay System	100 assays / 96-well plates 250 assays / 384-well plates	N2011
Nano-Glo® Live Cell Assay System	1.000 assays / 96-well plates 2.500 assays / 384-well plates	N2012
Nano-Glo® Live Cell Assay System	10.000 assays / 96-well plates 25.000 assays / 384-well plates	N2013

Detection of Luminescence and More...

GloMax® Discover is a high-performance, easy-to-use multimode plate reader for the detection of luminescence, fluorescence, UV/VIS absorbance, BRET, FRET and filtered luminescence. This high-performance instrument reads common plate formats (6-, 96- and 384-well) with very high sensitivity and over a broad linear range.

The system can be easily operated with an integrated tablet-PC and can be incorporated in an automated workflow.

GloMax DISCOVER

Various applications:

- Reporter gene assays
- Cell viability, cytotoxicity and apoptosis assays
- Kinetic measurements
- Multiplexing
- Assays for the detection of oxidative stress and cell metabolism
- ELISA
- BRET/FRET analysis

Please contact us to get personal advise at **promega_info@promega.com**

Worldwide Promega Contact Details

America

United States/Canada
Tel: 608-274-4330
Fax: 608-277-2516
Toll-Free Tel: 800 356 9526
Toll-Free Fax: 800 356 1970
E-mail: custserv@promega.com

Brazil
Tel: +55 11 5090 3780
Fax: +55 11 5096 3780
E-mail: promega.brasil@promega.com

Asia

China
Tel: +86 10 5825 6268
Fax: +86 10 5825 6160
Toll-Free: 800 810 8133
E-mail: info@promega.com.cn

Japan
Tel: 03 3669 7981
Fax: 03 3669 7982
E-mail: jpmktg@jp.promega.com

Korea
Tel: +82 2158 83718
Fax: +82 2262 85418
E-mail: CustServiceKR@promega.com

Pacific Asia Region, Singapore
Tel: +65 6513 3450
Fax: +65 6773 5210
E-mail: sg_custserv@promega.com

India
Tel: +91 11 43005814/15/16/17
Fax: +91 11 41035028
E-mail: ind_custserv@promega.com

Europe

Germany/Austria
Tel: +49 621 8501 291
Fax: +49 621 8501 222
E-mail: de_custserv@promega.com

Switzerland
Tel: +44 878 90 00
Fax: +44 878 90 10
E-mail: ch_custserv@promega.com

France
Tel: +33 0437 2250 00
Fax: +33 0437 2250 10
Numero Vert: 0 800 48 79 99
E-mail: contactfr@promega.com

Italy
Tel: +39 0254 0501 94
Fax: +39 0256 5616 45
Toll-Free Phone: 800 6918 18
E-mail: customerservice.italia@promega.com

Australia

Australia
Tel: 02 8338 3800
Fax: 02 8338 3855
Freecall: 1800 225123
Freefax: 1800 626 017
E-mail: auscustserv@promega.com

Poland
Tel: +48 22 531 0667
Fax: +48 22 531 0669
E-mail: pl_custserv@promega.com

Belgium/Luxembourg/
The Netherlands
Tel: +31 71 532 42 44
Fax: +31 71 532 49 07
E-mail: benelux@promega.com

Spain
Tel: +34 902 538 200
Fax:+34 902 538 300
E-mail: esp_custserv@promega.com

Denmark, Estonia, Finland,
Iceland, Norway, Sweden
Tel: +46 8 452 2450
Fax: +46 8 452 2455
E-mail: sweorder@promega.com

United Kingdom
Tel: +44 23 8076 0225
Fax: +44 23 8076 7014
Free Phone: 0800 378994
E-mail: ukcustserve@promega.com

For more information about Promega Branch Offices and Distributors visit:

www.promega.com/support/worldwide-contacts