A SEAP Reporter Gene System Selectable with Zeocin™
Catalog code: psetz-seap
https://www.invivogen.com/seap-reporter-gene

For research use only
Version 19J02-MM

PRODUCT INFORMATION
Contents
• 20 µg of pSELECT-zeo-SEAP plasmid provided as lyophilized DNA
• 1 ml of Zeocin™ (100 mg/ml)
• 1 ml of QB reagent and 1 ml of QB buffer (sufficient to prepare 100 ml of QUANTI-Blue™ Solution, a SEAP detection reagent).

Storage and Stability
• Product is shipped at room temperature. Lyophilized DNA should be resuspended upon receipt and stored at -20°C. Lyophilized DNA is stable for 3 months at -20°C. Resuspended DNA is stable more than one year at -20°C.
• Store Zeocin™ at 4°C or at -20°C. The expiry date is specified on the product label.
• QB reagent and QB buffer are stable for 1 year at -20°C. QUANTI-Blue™ Solution is stable for 2 weeks at 4°C and for 2 months at -20°C.

Quality control
• Plasmid construct has been confirmed by restriction analysis and sequencing.
• Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE
InvivoGen provides the secreted embryonic alkaline phosphatase (SEAP) gene in the pSELECT-zeo-SEAP plasmid. It can be used in vivo and in vitro to transfect mammalian cells stably or transiently. The SEAP gene expression is driven by the EF-1α/HTLV composite prom from the 5' untranslated region of the Human T-cell Leukemia Virus. The pSELECT-zeo-SEAP plasmid is selectable with Zeocin™ in both mammalian cells and bacteria.

SEAP is a reporter widely used to study promoter activity or gene expression. It is a truncated form of human placental alkaline phosphatase (PLAP) by deletion of the GPI anchor. Unlike endogenous alkaline phosphatases, PLAP is extremely heat stable and resistant to the inhibitor L-homoarginine. SEAP is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters. It allows to determine reporter activity without disturbing the cells, does not require the preparation of cell lysates and can be used for kinetic studies.

pSELECT-SEAP can serve as a gene reporter system for the study of eukaryotic gene expression and regulation.

PLASMID FEATURES
First expression cassette
• hEF1-HTLV prom is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter1 and the R segment and part of the U5 sequence (R-U5) of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat1. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene in vivo. The R-U5 has been coupled to the EF-1α core promoter to enhance stability of RNA.
• SEAP is a secreted form of human embryonic alkaline phosphatase. ORF size (from the ATG to the stop codon): 1563 bp
• SV40 pAn: the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
• ori: a minimal E. coli origin of replication to limit vector size, but with the same activity as the longer Ori.

Second expression cassette
• CMV enh/prom: The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
• EM7 is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in E. coli.
• Zeo: Resistance to Zeocin™ is conferred by the Sh ble gene from Streptocolloteichus hindustanus. The Sh ble gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and E. coli.
• ßGlo pAn: The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription.

METHODS
Plasmid resuspension
Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H2O. Store resuspended plasmid at -20°C.

Plasmid amplification and cloning
Plasmid amplification and cloning can be performed in E.coli GT116 or other commonly used laboratory E.coli strains, such as DH5α.

Zeocin™ usage
This antibiotic can be used for E. coli at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
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</thead>
<tbody>
<tr>
<td>QUANTI-Blue™ Solution</td>
<td>SEAP detection reagent</td>
<td>rep-qbs</td>
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<tr>
<td>Zeocin™</td>
<td>Selection antibiotic</td>
<td>ant-zn-1</td>
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TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com

www.invivogen.com
QUANTI-Blue™ Solution
Medium for detection and quantification of alkaline phosphatase in standard and HTS assays
Catalog code: rep-qbs, rep-qbs2
https://www.invivogen.com/quanti-blue
For research use only
Version 19F11-MM

PRODUCT INFORMATION
Contents
QUANTI-Blue™ Solution is available in two pack sizes:
- rep-qbs containing 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer to prepare 500 ml of QUANTI-Blue™ Solution sufficient for 25 x 96-well plates (standard procedure) or 20 x 1536-well plates (HTS screening)
- rep-qbs2 containing 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer to prepare 1 liter of QUANTI-Blue™ Solution sufficient for 50 x 96-well plates (standard procedure) or 40 x 1536-well plates (HTS screening)

Required Material (not provided)
- Sterile water
- Sterile screw-capped tube, glass bottle or flask

Storage and Stability
- Store QB reagent and QB buffer at -20°C. Product is stable for 1 year at -20°C when properly stored.
- Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8°C and for 2 months at -20°C. Protect QUANTI-Blue™ from light.

Quality Control
- Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.
- Physicochemical characterization (including pH, solubility).
- Functional assays using alkaline phosphatase or SEAP-expressing reporter cells.

DESCRIPTION
QUANTI-Blue™ is a colorimetric enzyme assay developed to determine any alkaline phosphatase activity (AP) in a biological sample, such as supernatants of cell cultures. QUANTI-Blue™ Solution changes from pink to a purple-blue color in the presence of AP. Secreted embryonic alkaline phosphatase (SEAP) is a widely used reporter gene. It is a truncated form of placental alkaline phosphatase, a GPI-anchored protein. SEAP is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters.

FEATURES AND ADVANTAGES
- Requires small samples of cell supernatants - 20 µl is sufficient.
- No need to process samples - Preparation of cell lysates or heating of samples is not required.
- Determine secreted AP activity without disturbing cells - The same cell cultures can be repeatedly sampled for kinetic studies.
- Assay can be completed in 30 min - Hands-on time no longer than 10 min. The enzymatic activity can be detected as early as 15 min after incubation of the samples in QUANTI-Blue™ Solution.
- Wide dynamic range allows to detect low and high levels of AP - No need to perform multiple sample dilutions.
- Highly sensitive for quantitative measurement - Higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity.
- Extremely simple to use - 1) Prepare solution with water, 2) add sample to detection reagent, 3) incubate at 37°C, and 4) assess AP activity.

METHODS
QUANTI-Blue™ Solution has been optimized for use in 96-well plates (standard procedure) and in 1536-well plates (high throughput screening procedure).

A. Standard procedure
Figure 1. Standard procedure using QUANTI-Blue™ Solution.
The following protocol refers to the use of 96-well plates. Ensure QB reagent and QB buffer are completely thawed before use.

Note: For fast thawing, QB reagent and QB buffer can be placed at 37°C for 2 minutes. Ensure heating at 37°C does not exceed 5 minutes.

1. Prepare 100 µl of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 98 µl of sterile water in a sterile glass bottle or flask.
2. Mix well by vortexing and incubate at room temperature for 10 min before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8°C or -20°C.
4. Dispense 180 µl of QUANTI-Blue™ Solution per well into a flat-bottom 96-well plate.
5. Add 20 µl of sample (supernatant of SEAP-expressing cells) or negative control (cell culture medium).
6. Incubate at 37°C for 15 min to 6 h.
7. Measure optical density (OD) at 560-590 nm using a microplate reader.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend to heat FBS at 56°C for 30 min to inactivate the alkaline phosphatase activity.

For different cell culture plate formats, please refer to the table below:

<table>
<thead>
<tr>
<th></th>
<th>96-well plate</th>
<th>24-well plate</th>
<th>12-well plate</th>
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<tbody>
<tr>
<td>QUANTI-Blue™</td>
<td>180 µl</td>
<td>450 µl</td>
<td>900 µl</td>
</tr>
<tr>
<td>Supernatant</td>
<td>20 µl</td>
<td>50 µl</td>
<td>100 µl</td>
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TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com
### B. High Throughput Screening (HTS) procedure

**Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.**

This procedure has been optimized for use in HTS screening procedures in 1536-well plates. QUANTI-Blue™ Solution is added directly to the cell suspension to reduce liquid handling. Ensure QB reagent and QB buffer are completely thawed before use.

**Note:** For fast thawing, QB reagent and QB buffer can be placed at 37 °C for 2 minutes. Ensure heating at 37 °C does not exceed 5 minutes.

1. Dispense cell suspension and test compounds into a 1536-well plate in a volume that does not exceed 5 µl per well. Incubate cells with test compounds for the desired period of time. Ensure heating at 37 °C does not exceed 5 minutes.
2. Prepare 17 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 15 ml of sterile H2O in a 50 ml screw cap tube.
3. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
4. Use QUANTI-Blue™ Solution immediately or store at 2-8 °C or -20 °C.
5. Dispense 2 µl of QUANTI-Blue™ Solution to the wells containing ≤5 µl of cell culture in a 1536-well plate.
6. Incubate at 37°C for 15 min to 6 hr.
7. Measure OD at 620-655 nm.

**Note:** If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend to heat FBS at 56°C for 30 min to inactivate the alkaline phosphatase activity.

### RELATED PRODUCTS

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<tr>
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<tr>
<td>pNiFty2-SEAP (Zeo+)</td>
<td>pnifty2-seap</td>
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<tr>
<td>pSELECT-zeo-SEAP</td>
<td>pselect-seap</td>
</tr>
<tr>
<td>HEK-Blue™ Detection</td>
<td>hb-det2</td>
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<tr>
<td>Recombinant SEAP Protein</td>
<td>rec-hseap</td>
</tr>
</tbody>
</table>

**Reporter cells**

- HEK-Blue™ hTLR2: hkb-htlr2
- HEK-Blue™ hTLR4: hkb-htlr4
- RAW-Blue™ Cells: raw-sp
- THP1-Blue™ NF-κB Cells: thp-nfk
- THP1-Blue™ ISG Cells: thp-isg

For a complete list of InvivoGen's Reporter Cell Lines visit [https://www.invivogen.com/reporter-cells](https://www.invivogen.com/reporter-cells)