

QUANTI-Blue™

Medium for detection and quantification of alkaline phosphatase

Catalog code: rep-qb1, rep-qb2

<https://www.invivogen.com/quanti-blue>

For research use only

Version 18L10-MM

PRODUCT INFORMATION

Contents

QUANTI-Blue™ is provided as packs of individually sealed pouches.

- **rep-qb1:** 5 pouches of QUANTI-Blue™, sufficient to prepare 500 ml of QUANTI-Blue™ medium

- **rep-qb2:** 10 pouches of QUANTI-Blue™, sufficient to prepare 1 liter of QUANTI-Blue™ medium

Required Material (not provided)

- Sterile water
- Sterile glass bottle or flask

Storage and Stability

- Store QUANTI-Blue™ pouches at 2-8°C. Product is stable for 1 year when properly stored.
- Reconstituted QUANTI-Blue™ medium is stable for 2 weeks at 2-8°C and for 2 months at -20°C. Protect reconstituted 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™ medium 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

The following protocol refers to the use of 96-well plates.

1. Pour the contents of one pouch of QUANTI-Blue™ in a 250 ml sterile glass bottle or flask.
2. Add 100 ml of sterile water. Swirl gently.
3. Warm QUANTI-Blue™ medium to 37°C for 30 min.
4. Use QUANTI-Blue™ medium immediately or store at 2-8°C or -20°C.
5. Dispense 180 µl of QUANTI-Blue™ medium per well into a flat-bottom 96-well plate.
6. Add 20 µl of sample (supernatant of SEAP-expressing cells) or negative control (cell culture medium).
7. Incubate at 37°C for 15 min to 6 h.
8. Measure optical density (OD) at 620-655 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:

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	180 µl	450 µl	900 µl
Supernatant	20 µl	50 µl	100 µl

For your convenience, InvivoGen also provides QUANTI-Blue™ in liquid form. QUANTI-Blue™ Solution is available in two pack sizes:

- **rep-qbs:** 5 x 1 ml of QB reagent + 5 x 1 ml QB buffer, sufficient to prepare 500 ml of QUANTI-Blue™ Solution
- **rep-qbs2:** 10 x 1 ml of QB reagent + 10 x 1 ml QB buffer, sufficient to prepare 1 liter of QUANTI-Blue™ Solution

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ Detection	hb-det2
QUANTI-Blue™ Solution	rep-qbs
pNifty2-SEAP (Zeo®)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
Recombinant SEAP Protein	rec-hseap

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