PeptiMatrix™
Next generation peptide hydrogels

STANDARD OPERATING PROCEDURE

PRESTOBLUE™ ASSAY FOR CELL VIABILITY OF CELLS IN PEPTIMATRIX™ HYDROGELS

1. BACKGROUND

Cell viability is a key indicator of cell health. PrestoBlue™ is a quick cell viability indicator that relies on live cells to convert resazurin to the fluorescent molecule, resorufin. The fluorescence signal measured can be evaluated quantitatively on an absorbance or fluorescence-based microplate reader; while qualitative analysis can be evaluated by the visual colour change of the solution which is indicative of metabolically active cells. This protocol was adapted from the manufacturer's protocol.

2. RISK ASSESSMENT

Always follow your organisation's laboratory safety procedures.

Work inside an appropriate microbiology safety cabinet for your cell type. Refer to the **PeptiMatrix Safety Data Sheet (SDS)** for detailed safety, handling, storage, and first aid information relating to the hydrogel components.

If you are working with additional cell lines, media supplements, matrix additives, or other reagents, consult the relevant SDS documents for those materials as well.

3. MATERIALS

- Class II microbiology safety cabinet (or appropriate class for your cell type)
- Cells encapsulated or seeded on PeptiMatrix hydrogels
- P1000, P200 pipettes and filter tips
- 96-well plate with cells growing
- PrestoBlue Cell Viability Reagent, (ThermoFisher, A13261)
- Phosphate-buffered saline (PBS) (ThermoFisher, 14190-144)
- Fluorescence or absorbance microplate reader

4. METHODS

4.1 Reagent preparation

This procedure can be scaled up or down depending on the number of wells you plan to prepare, and which well plate size you are using.

1. Prepare the PrestoBlue working solution as follows:

    - **Cells on top of gels:** dilute 10 µL PrestoBlue reagent in 90 µL cell culture medium per well (1:10).
    - **Cells encapsulated within gels:** dilute 10 µL PrestoBlue reagent in 40 µL cell culture medium per well (1:5). This assumes 100 µL gel and cell suspension per well in a 96 well plate. After adding 100 µL of the 1:5 PrestoBlue solution to the gel, the final reagent concentration matches the 1:10 dilution used for monolayer cultures.

    When using a 96 well plate, multiply volumes by the number of wells. For example, for 10 wells using a 1:10 dilution, mix 100 µL reagent with 900 µL medium. For other plate formats, refer to the table below.

Format	Volume of medium	Volume of PrestoBlue reagent
Cuvette	900 µL	100 µL
96-well plate	90 µL	10 µL
384-well plate	36 µL	4 µL
1,536-well plate	5 µL	3 µL

4.2 PrestoBlue™ cell viability protocol

1. Remove cell media from wells and wash gels once DPBS.
2. Add the diluted reagent to the wells, 100 µL per well.
3. Incubate the plate in a cell culture incubator at 37 °C for 10 minutes. For cells encapsulated within gels, a longer incubation time of up to 1 hour may be preferable
4. Read fluorescence or absorbance on a suitable plate reader. Excitation/Emission for fluorescence reading should be 560/590 nm (or closest values possible). While absorbance should be measured at <570 nm (use 600 nm reference wavelength).

4.2 Protocol tips

- Correct for background fluorescence by including control wells containing only cell culture medium (no cells) on each plate.
- Fluorescence is more sensitive than absorbance and is the preferred detection method.
- Bottom-read is more sensitive than top-read.

5. DISPOSAL

Dispose of hydrogels containing cells, media, or matrix components according to your local guidelines for biological waste.

6. DOCUMENT HISTORY

Version	Date	Summary of Changes
1.0	**05 Feb 26**	First version of customer facing SOP, adapted from internal PeptiMatrix PrestoBlue procedures.