SOP TITLE:	VERSION:	
PM-PD-SOP3.3 PrestoBlue assay for cell viability	1	
AUTHOR & RESEARCH TEAM:	STATUS:	
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SOP REVIEWED AND APPROVED BY VJE (SEP 19, 2024, 3:25 PM +0100)

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1. BACKGROUND

PrestoBlue® is a ready to use cell permeable resazurin-based solution that functions as a cell viability indicator by using the reducing power of living cells to quantitatively measure the proliferation of cells. When added to cells, the PrestoBlue® reagent is modified by the reducing environment of the viable cell and turns red in color, becoming highly fluorescent. This color change can be detected using fluorescence or absorbance measurements.

2. RISK ASSESSMENT

Please refer to PM-RA0 and PM-RA2 for a full risk assessment of the following protocol. Ensure that you fully understand the potential risks and have received the appropriate training before starting any work.

3. MATERIALS

- PrestoBlue® Cell Viability Reagent (Invitrogen)
- Plate reader with fluorescence probe (e.g., FLUOstar OPTIMA, UK/ Infinite® F50, UK)
- Class II Microbiological Safety Cabinet
- Cells for staining in 96-well plate (preferably opaque walled with optical polymer base)
- P200 filter tips
- P200 pipette

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Product	Supplier	Cat No	Storage

PrestoBlue® Cell Viability Reagent	Invitrogen	A13261	-4°C and protect from light

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4. METHODS

Preparation of 2X PrestoBlue® staining solution

1. Dilute PrestoBlue® Cell Viability Reagent (10X) to 2X by diluting in cell culture media.

Staining gels in 96-well plate

1. Remove media from the top of the gel.
2. Add an equal volume of 2X PrestoBlue® staining solution for the volume of gel in each well (e.g., for 100 µL of gel, add 100 µL of staining solution).
    1. This will dilute the 2X PrestoBlue® staining solution to 1X.
    2. Also consider using 1-2 wells with no cells (gel + media only) to correct for any background fluorescence later.
3. Incubate plate in the dark (cover with foil) for 10 min at 37 °C.
4. Measure absorbance at Excitation/Emission 560/590 nm using a plate reader.

Staining gels in a cell culture insert

For cells grown in gels plated out in a 24-well plate cell culture insert, the same basic procedure as above can be used, but you may need to first remove the gels from the cell culture insert and transfer them to a 96-well plate prior to staining:

1. Remove as much media as possible from the top of the gel.
2. Transfer insert and place on top of paper towel to blot excess media from bottom.
3. Using a scalpel, and holding the insert horizontally, carefully score around the membrane on the bottom of the insert.
4. Remove membrane with tweezers.
5. Transfer gel to 96-well plate by placing the insert vertically over a single well.
    1. In most cases, the gel should slide out of the insert and into the well.
    2. If the gel does not slide out, you can use the flat end of a P10 pipette tip to slowly push the gel from the top into the well.
6. Add an equal volume of 2X PrestoBlue® staining solution to the volume of gel in each well (e.g., for 100 µL of gel, add 100 µL of staining solution).
    1. This will dilute the 2X PrestoBlue® staining solution to 1X.
    2. Also consider using 1-2 wells with no cell (gel + media only) to correct for any background fluorescence later.
7. Incubate plate in the dark (cover with foil) for 10 min at 37 °C.
8. Measure absorbance at Excitation/Emission 560/590 nm using a plate reader.

5. DISPOSAL

1. Dispose of cell culture medium, and any solutions that have been in contact with cells, by pouring into waste pot/ aspirating into vacuum trap containing Chemgene. Leave in Chemgene for at least one hour.
2. Dispose of any unneeded glass in appropriate glass bins.
3. Dispose of contaminated sharps in appropriate sharps bins.

User Initials Legend

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