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Some adherent cells can be used directly on the BD™ Oxygen Biosensor System (BD™ OBS) because they produce their own extracellular matrix in sufficient quantity. For cell lines lacking this ability, it becomes necessary to present the cells with an anchorage-friendly surface. We have found that common, commercially available micro-carrier beads, such as Cytodex™-3, work nicely for this purpose. The recommended approach entails addition of prepared Cytodex beads to the plate just prior to adding the cells. This is simpler and more straightforward than culturing the cells on the beads. To demonstrate this protocol, 3T3 cells were used as an example. The protocol may be adapted as appropriate for other adherent cell lines.

Growth media for 3T3 cells

Dulbecco's Modified Eagle Medium
(GibcoBRL Cat. No. 11885-084)

The following were added to the medium with final concentrations below:

- Glucose increased to 4.5 g/L
- 10% FBS
- 1x MEM Non Essential Amino Acids (GibcoBRL Cat. No. 11140-050)
- 25 mM HEPES (GibcoBRL Cat. No. 15630-080)

The cells were grown in BD Falcon™ 175 cm² Tissue Culture Flasks (Cat. No. 353112) and harvested before they reached confluency. The cells were dissociated with Trypsin and diluted as required in the above growth media.

Preparation of Cytodex-3 Beads

1. Cytodex-3 beads were suspended and hydrated for two hours in sterile GibcoBRL Dulbecco's Phosphate Buffered Saline (Cat. No. 14190-136). There are 3 x 10⁶ beads per gram of dry weight.
2. Beads were sterilized by autoclaving for 15 minutes at 115°C and 15 psi, and were stored at 4°C until use.
3. On the day of use, the beads were spun at 1,000 rpm for five minutes. The buffer was removed and 37°C warmed growth media was added to the beads. This wash step was done twice.
4. The beads were diluted to 120,000 beads/ml in the growth media.
5. For more information about Cytodex beads, see the Amersham Biosciences website, www.amershambiosciences.com

Addition of Cytodex-3 and 3T3 to plate

The BD OBS was prepared by performing the following steps:

1. Add 50 µl of beads to each well (approximately 6,000 beads per well). To ensure uniform suspension of the beads, the reservoir was continuously shaken while pipetting the beads.
2. Add 150 µl of cell suspension at various dilutions.
3. Incubate the plate at 37°C, 5% CO₂. When readings were to be made, the plates were transferred from the incubator to the fluorescence reader with minimal time lag to prevent temperature change.
4. The plates were read periodically over several days to follow the proliferation of the cells.
5. For guidance on plate reader settings and data work-up, please see the *Guidelines for Use of the BD Oxygen Biosensor System* located on our website.

For additional information on the BD™ Oxygen Biosensor System, visit our website at:

www.bdbiosciences.com/discovery_labware/Products/drug_discovery/oxygen_biosensor_system

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