TEST CITOTOSSICITÀ

**Materials**

Cell line INS

Medium INS

Multiwell 96

PBS

CaCl2 solution

Hydrogel formulation. Alginate (2.5%) + CMC (1%). Solvent HBSS. p/s (1%), amp (1%), HEPES (10mM)

**Protocol**

**DAY 1**

1. Prepare hydrogel with Alginate (2.5%) + CMC (1%); HBSS, p/s, amp (1%), HEPES
2. We will have 2 vials each one containing 2 ml of hydrogel formulation
3. Preparation of the CaCl2 solutions: 0.1 M, 0.05 M, 0.02 M, 0.01 M.

**DAY 3**

Filter CaCl2 solutions (with 0.45 um filters)

1. Prepare a water bath at 37 °C for hydrogel solution (keep warm).
2. Split cells and resuspend in 3ml after the centrifuge (if confluence is high)
3. Counting them
4. Centrifuge again and then resuspend cells in 20-50 ul
5. Put cells in each vials (each vial contains 2 ml of hydrogel).
6. Put vials in incubator for 5 minutes with slow rotation

**Unvaried time with different concentrations (18 wells)**

For 96 multi-well pour 66.33 ul in each well. Put inside 200 ul of CaCl2 solutions in each well for 10 minutes. Four solutions with four different concentrations. After 10 min remove the crosslinking solution, wash twice with PBS and add 200 ul of INS medium.

Old one (Remove the medium and wash the little well with 400 ul of PBS. Now pour the 400 ul of CaCl2 solutions in each well for 10 minutes. There are four solutions each one of different concentrations. After 10 minutes remove the solutions. Now wash again with 400 ul of PBS and refill with the same amount of medium.)

**Unvaried concentration with variable time (21 wells)**

For 96 multi-well Pour 66.33 ul of hydrogel in each well. In this experiment we put inside one type of solution 0.1M for different time. Remove the solutions in chronological order. Remove it after 1 minute, 2 minutes, 5 minutes, 10 minutes, 20 minutes. Wash twice with PBS and add 200 ul medium.

**Unvaried time with different concentrations (18 wells)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **10 min** | **10 min** | **10 min** | **10 min** |
| **0,1 M** |  |  |  |  |
| **0,05 M** |  |  |  |  |
| **0,02 M** |  |  |  |  |
| **0,01 M** |  |  |  |  |
| **Gel control** |  |  |  |  |
| **Cell control** |  |  |  |  |

**Unvaried concentration with variable time (21 wells)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **0,1 M** | **0,1 M** | **0,1 M** | **0,1 M** |
| **1 MIN** |  |  |  |  |
| **2 MIN** |  |  |  |  |
| **5 MIN** |  |  |  |  |
| **10 MIN** |  |  |  |  |
| **20 MIN** |  |  |  |  |
| **Gel control** |  |  |  |  |
| **Cell control** |  |  |  |  |