A common European approach to the regulatory testing of nanomaterials

Standard Operating Procedure

Title	***Viability protocol by using a Cell Counter***
Subtitle	
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2		
3		
4		

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1 Materials

- Desired cells
- PBS
- PBS + 0.1% FBS
- Trypsin (in case of adherent cultures)
- Cell culture medium
- Beckman-Coulter Cell Counter

2 Procedure

1) In the case of adherent cultures, trypsinize and resuspend the cells in a known volume of PBS + 0.1% FBS. The volume of trypsin, its concentration and the trypsinization time depends on the cell line. Cells that grow in suspension can be counted directly.
2) Prepare a cuvette with 9.9 mL of PBS and add 100 µL of your cell culture (1:100 dilution).
3) Set the Coulter Counter:
   a. Set the dilution factor to 1 E + 02.
   b. Set the approximate size of the cells, entering the lower limit (Tl) and higher (You).
4) Shake the sample without making bubbles. Place the electrode tube inside the cuvette.
5) Press start. When the machine has finished, press "output" and manually adjust your cells' size to refine the count. This gives you the amount of cell/mL. Multiply this value for the volume you have, to get the total number of cells you have in your cell culture.