The colony forming efficiency and alamarBlue assays: Principles and applications

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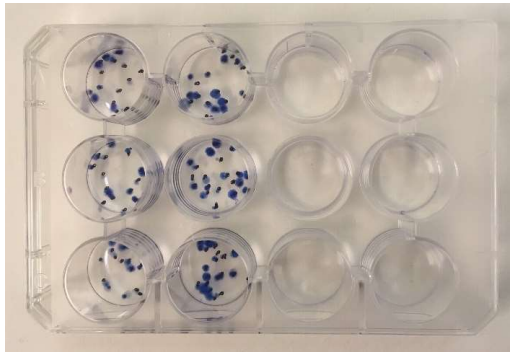
Colony forming efficiency assay (CFE)

- Percentage of cells inoculated at a low density that give rise to colonies
  - Also known as plating efficiency
- Interference free assay for testing cytotoxicity of chemicals and particles
- Can also be used for comparing size of the colonies
  - E.g. growth inhibition or growth fascilitation



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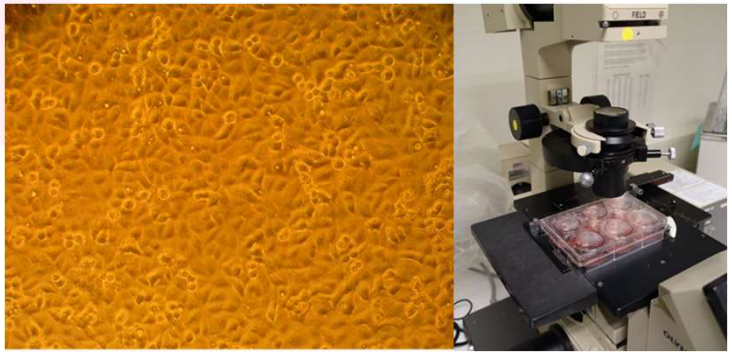
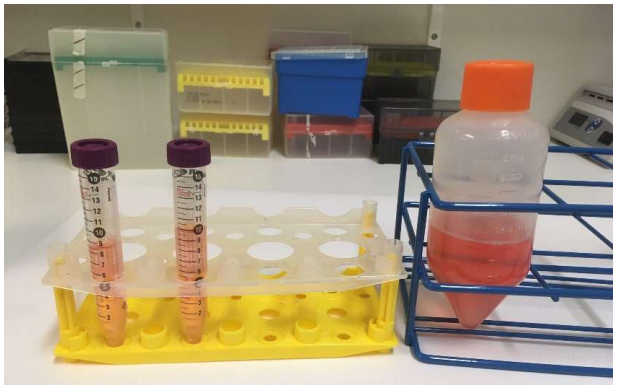
Colony forming efficiency assay

Detach cells by trypsine

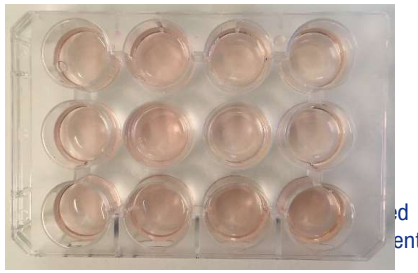


Dilute cells to requested concentration



Add cells diluted in medium.
Wait for at least one hour

Add substances
E.g, 6 wells per concentrations



Let stay for
approx 10 days

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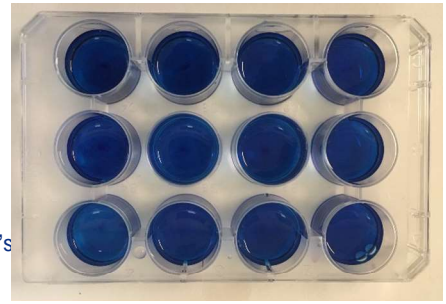
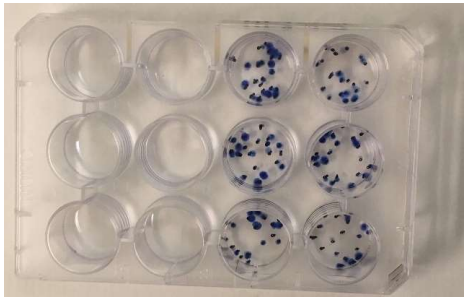
Stain cells with methylene blue

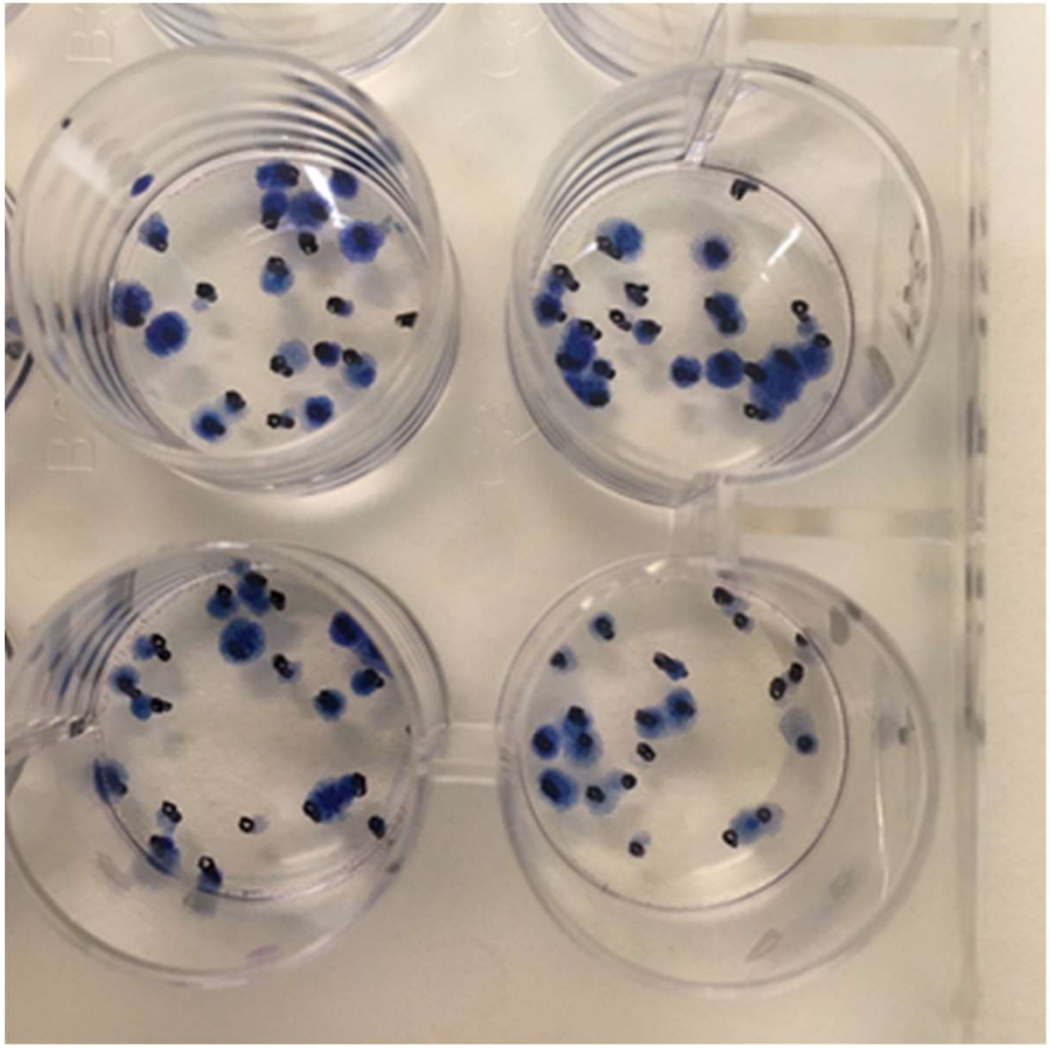


Remove staining and count colonies



Counting of colonies

- At least 50 cells per colony
  - Visible bye eye implies more than 50 colonies
- Some colonies are very close to each other
  - As long as you count the colonies in the same way it does not matter
- Compare effect relative to control



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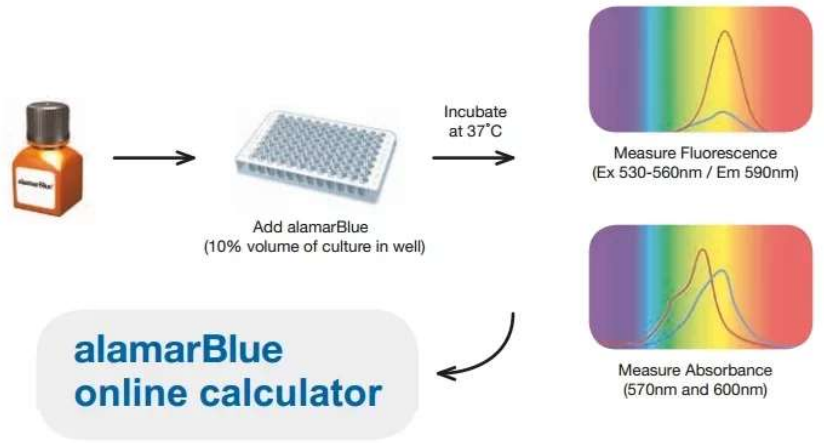
AlamarBlue cytotox assay

- Cell viability assay
  - Uses the blue dye resazurin (weakly fluorescent)
  - Measures cell proliferation
- Resazurin is cell permeable
  - In cells it can be reduced by components in the electron transport chain
  - Resazurin is reduced to the strongly fluorescent resorufin (red/pink colour)
- Fluoresent substances may interfere with test substances
  - Must be tested



From Bio-Rad

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AlamarBlue cytotox assay, procedure

- Prepare 10% alamarBlue reagence in cell culture medium
- Remove growth medium from the cells
- Wash the cells with prewarmed PBS
  - Can be optional, but recommended if exposed to particles
- Add the alamarBlue mix to the cells
  - e.g. 100-200 µl to wells in 96 plates
- Let stay in incubator for 1-4 hours
- Take aliquotes from the AB-exposed cells and measure the fluorescence or absorbance in a plate reader.
- Subtract background fluorescence (AB-mix only) and compare to the negative controls.

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THANK YOU!

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