# Protocol for Flow Cytometer for 96 Well plates

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## Step 1: Diluting the liquid cultures

1. Measure the OD600 of the liquid culture using the spectrophotometer.
2. Dilute the liquid culture so that the final OD600 is 0.2 (4x108cells/ml). There should be 4ml of the diluted liquid culture.
3. Conduct a series dilution by pipetting 400μl of the diluted liquid culture and adding 3600μl of filtered marine broth into a 15mL falcon tube. Pipette to mix well. Repeat this step until you reach a CFU of 4x100cells/ml.
4. You should have 10 falcon tubes containing 3.6ml/4ml of liquid culture including the blank.

## Step 2: Preparing the 96 well plate

1. Transfer 200μl of the unstained solution into each of the wells according to the green section of the table below.
2. The following steps are to be carried out in the darkroom:
3. Pipette 1800μl of the diluted liquid culture into a 2mL Eppendorf tube and add 1.8μl of DAPI into it.

\*Note the ratio of DAPI to medium is 1μl:1mL

1. Transfer 200μl of the DAPI-stained solution into each of the wells according to the blue section of the table below. (There should be 1.2ml of DAPI-stained medium left in the Eppendorf tube.)
2. Add 1.8/2.2μl of STY09 into falcon tubes containing 1.8/2.2mL of liquid culture.

\*Note the ratio of STY09 to medium is 1μl:1mL

1. Transfer 200μl of the STY09 -stained solution into each of the wells according to the orange section of the table below. (There should be 1.2/1.6ml of STY09 stained medium left in the falcon tube.)

Plate 1

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 4x108 | 4x107 | 4x106 | 4x105 | 4x104 | 4x103 | 4x102 | 4x101 | 4x100 | Blank |  |  |
| B | 4x108 | 4x107 | 4x106 | 4x105 | 4x104 | 4x103 | 4x102 | 4x101 | 4x100 | Blank |  |  |
| C | 4x108 | 4x107 | 4x106 | 4x105 | 4x104 | 4x103 | 4x102 | 4x101 | 4x100 | Blank |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E | 4x108 | 4x107 | 4x106 | 4x105 | 4x104 | 4x103 | 4x102 | 4x101 | 4x100 | Blank |  |  |
| F | 4x108 | 4x107 | 4x106 | 4x105 | 4x104 | 4x103 | 4x102 | 4x101 | 4x100 | Blank |  |  |
| G | 4x108 | 4x107 | 4x106 | 4x105 | 4x104 | 4x103 | 4x102 | 4x101 | 4x100 | Blank |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

Plate 2

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 4x108 | 4x107 | 4x106 | 4x105 | 4x104 | 4x103 | 4x102 | 4x101 | 4x100 | Blank |  |  |
| B | 4x108 | 4x107 | 4x106 | 4x105 | 4x104 | 4x103 | 4x102 | 4x101 | 4x100 | Blank |  |  |
| C | 4x108 | 4x107 | 4x106 | 4x105 | 4x104 | 4x103 | 4x102 | 4x101 | 4x100 | Blank |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |  |  |  |  |  |
| F |  |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

Legend:

|  |  |  |  |
| --- | --- | --- | --- |
|  | | Unstained | |
|  | | STY09 | |
|  | | DAPI | |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | 11 | | 12 | |
| A | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | |  | |  | |
| B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | |  | |  | |
| C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | |  | |  | |
| D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | |  | |  | |
| E | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | |  | |  | |
| F | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | |  | |  | |
| G | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | |  | |  | |
| H |  |  |  |  |  |  |  |  |  |  | |  | |  | |