

Multicolor fluorescence per bacterial particle calibration

Description:

Plate readers report fluorescence values in arbitrary units that vary widely from instrument to instrument. Therefore absolute fluorescence values cannot be directly compared from one instrument to another. In order to compare fluorescence output of biological devices, it is necessary to create a standard fluorescence curve. This variant of the protocol uses two replicates of three colors of dye, plus beads.

Adapted from <https://dx.doi.org/10.17504/protocols.io.bht7j6rn> and <https://dx.doi.org/10.17504/protocols.io.6zrhf56>

Protocol Inputs:

Materials

- * [Water, sterile-filtered, BioReagent, suitable for cell culture] (<https://identifiers.org/pubchem.substance:24901740>)
- * 10 uM [fluorescein] (<https://identifiers.org/pubchem.substance:329753341>) in PBS
- * [Phosphate-Buffered Saline] (<https://identifiers.org/pubchem.compound:24978514>)
- * 10 uM [cascade blue] (<https://identifiers.org/pubchem.substance:329760049>) in ddH2O
- * 2 uM [sulforhodamine 101] (<https://identifiers.org/pubchem.substance:24899749>) in PBS
- * 3e9 microspheres/mL ddH2O (NanoCym 950 nm monodisperse silica nanoparticles)

Containers

- * [Calibration Plate] (<https://identifiers.org/ncit:C43377>) ([Microplate] (<https://identifiers.org/ncit:C43377>))
- * [Fluorescein] (<https://identifiers.org/ncit:C50236>) ([Tube Device] (<https://identifiers.org/ncit:C50236>))
- * [Cascade Blue] (<https://identifiers.org/ncit:C50236>) ([Tube Device] (<https://identifiers.org/ncit:C50236>))
- * [Silica beads] (<https://identifiers.org/ncit:C50236>) ([Tube Device] (<https://identifiers.org/ncit:C50236>))
- * [Sulforhodamine 101] (<https://identifiers.org/ncit:C50236>) ([Tube Device] (<https://identifiers.org/ncit:C50236>))
- * [Liquid Waste Disposal] (<https://identifiers.org/ncit:C48166>) ([Disposal] (<https://identifiers.org/ncit:C48166>))

Steps

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1. Pipette 1.0 milliliter of [10 uM fluorescein in PBS] (<https://identifiers.org/SBO:0000241>) into [Fluorescein] (<https://identifiers.org/ncit:C50236>)

2. Pipette 1.0 milliliter of [2 uM sulforhodamine 101 in PBS] (<https://identifiers.org/SBO:0000241>) into [Sulforhodamine 101] (<https://identifiers.org/ncit:C50236>)
3. Pipette 1.0 milliliter of [10 uM cascade blue in ddH2O] (<https://identifiers.org/SBO:0000241>) into [Cascade Blue] (<https://identifiers.org/ncit:C50236>)
4. Pipette 1.0 milliliter of [3e9 microspheres/mL ddH2O] (<https://identifiers.org/SBO:0000241>) into [Silica beads] (<https://identifiers.org/ncit:C50236>)
5. Pipette 100.0 microliter of [Phosphate-Buffered Saline] (<https://identifiers.org/pubchem.compound:24978514>) into [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A2:D12
6. Pipette 100.0 microliter of [Water, sterile-filtered, BioReagent, suitable for cell culture] (<https://identifiers.org/pubchem.substance:24901740>) into [Calibration Plate] (<https://identifiers.org/ncit:C43377>) E2:H12
7. Pipette 200.0 microliter of [10 uM fluorescein in PBS] (<https://identifiers.org/SBO:0000241>) from [Fluorescein] (<https://identifiers.org/ncit:C50236>) into [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A1:B1
8. Pipette 100.0 microliter from [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A1:B1 into [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A2:B2, mixing by pipetting up and down 3.0 times at destination
9. Pipette 100.0 microliter from [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A2:B2 into [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A3:B3, mixing by pipetting up and down 3.0 times at destination
10. Pipette 100.0 microliter from [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A3:B3 into [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A4:B4, mixing by pipetting up and down 3.0 times at destination
11. Pipette 100.0 microliter from [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A4:B4 into [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A5:B5, mixing by pipetting up and down 3.0 times at destination
12. Pipette 100.0 microliter from [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A5:B5 into [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A6:B6, mixing by pipetting up and down 3.0 times at destination
13. Pipette 100.0 microliter from [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A6:B6 into [Calibration Plate] (<https://identifiers.org/ncit:C43377>) A7:B7, mixing by pipetting up and down 3.0 times at destination

14. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) A7:B7 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) A8:B8, mixing by pipetting up
and down 3.0 times at destination
15. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) A8:B8 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) A9:B9, mixing by pipetting up
and down 3.0 times at destination
16. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) A9:B9 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) A10:B10, mixing by pipetting up
and down 3.0 times at destination
17. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) A10:B10 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) A11:B11, mixing by pipetting up
and down 3.0 times at destination
18. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) A11:B11 to [Liquid Waste
Disposal] (<https://identifiers.org/ncit:C48166>)
19. Pipette 200.0 microliter of [2 uM sulforhodamine 101 in PBS]
(<https://identifiers.org/SBO:0000241>) from [Sulforhodamine 101]
(<https://identifiers.org/ncit:C50236>) into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C1:D1
20. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C1:D1 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C2:D2, mixing by pipetting up
and down 3.0 times at destination
21. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C2:D2 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C3:D3, mixing by pipetting up
and down 3.0 times at destination
22. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C3:D3 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C4:D4, mixing by pipetting up
and down 3.0 times at destination
23. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C4:D4 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C5:D5, mixing by pipetting up
and down 3.0 times at destination
24. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C5:D5 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C6:D6, mixing by pipetting up
and down 3.0 times at destination
25. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C6:D6 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C7:D7, mixing by pipetting up

and down 3.0 times at destination

26. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C7:D7 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C8:D8, mixing by pipetting up
and down 3.0 times at destination

27. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C8:D8 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C9:D9, mixing by pipetting up
and down 3.0 times at destination

28. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C9:D9 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C10:D10, mixing by pipetting up
and down 3.0 times at destination

29. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C10:D10 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C11:D11, mixing by pipetting up
and down 3.0 times at destination

30. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C11:D11 to [Liquid Waste
Disposal] (<https://identifiers.org/ncit:C48166>)

31. Pipette 200.0 microliter of [10 uM cascade blue in ddH2O]
(<https://identifiers.org/SBO:0000241>) from [Cascade Blue]
(<https://identifiers.org/ncit:C50236>) into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E1:F1

32. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E1:F1 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E2:F2, mixing by pipetting up
and down 3.0 times at destination

33. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E2:F2 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E3:F3, mixing by pipetting up
and down 3.0 times at destination

34. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E3:F3 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E4:F4, mixing by pipetting up
and down 3.0 times at destination

35. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E4:F4 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E5:F5, mixing by pipetting up
and down 3.0 times at destination

36. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E5:F5 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E6:F6, mixing by pipetting up
and down 3.0 times at destination

37. Pipette 100.0 microliter from [Calibration Plate]

(<https://identifiers.org/ncit:C43377>) E6:F6 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E7:F7, mixing by pipetting up
and down 3.0 times at destination

38. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E7:F7 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E8:F8, mixing by pipetting up
and down 3.0 times at destination

39. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E8:F8 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E9:F9, mixing by pipetting up
and down 3.0 times at destination

40. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E9:F9 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E10:F10, mixing by pipetting up
and down 3.0 times at destination

41. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E10:F10 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E11:F11, mixing by pipetting up
and down 3.0 times at destination

42. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E11:F11 to [Liquid Waste
Disposal] (<https://identifiers.org/ncit:C48166>)

43. Pipette 200.0 microliter of [3×10^9 microspheres/mL ddH₂O]
(<https://identifiers.org/SBO:0000241>) from [Silica beads]
(<https://identifiers.org/ncit:C50236>) into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G1:H1

44. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G1:H1 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G2:H2, mixing by pipetting up
and down 3.0 times at destination

45. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G2:H2 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G3:H3, mixing by pipetting up
and down 3.0 times at destination

46. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G3:H3 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G4:H4, mixing by pipetting up
and down 3.0 times at destination

47. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G4:H4 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G5:H5, mixing by pipetting up
and down 3.0 times at destination

48. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G5:H5 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G6:H6, mixing by pipetting up
and down 3.0 times at destination

49. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G6:H6 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G7:H7, mixing by pipetting up
and down 3.0 times at destination
50. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G7:H7 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G8:H8, mixing by pipetting up
and down 3.0 times at destination
51. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G8:H8 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G9:H9, mixing by pipetting up
and down 3.0 times at destination
52. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G9:H9 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G10:H10, mixing by pipetting up
and down 3.0 times at destination
53. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G10:H10 into [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G11:H11, mixing by pipetting up
and down 3.0 times at destination
54. Pipette 100.0 microliter from [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G11:H11 to [Liquid Waste
Disposal] (<https://identifiers.org/ncit:C48166>)
55. Measure fluorescence of [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) A1:B12 at excitation 488.0
nanometer and emission 530.0 nanometer / 30.0 nanometer
56. Measure fluorescence of [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) C1:D12 at excitation 561.0
nanometer and emission 610.0 nanometer / 20.0 nanometer
57. Measure fluorescence of [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) E1:F12 at excitation 405.0
nanometer and emission 450.0 nanometer / 50.0 nanometer
58. Measure absorbance of [Calibration Plate]
(<https://identifiers.org/ncit:C43377>) G1:H12 at 600.0 nanometer