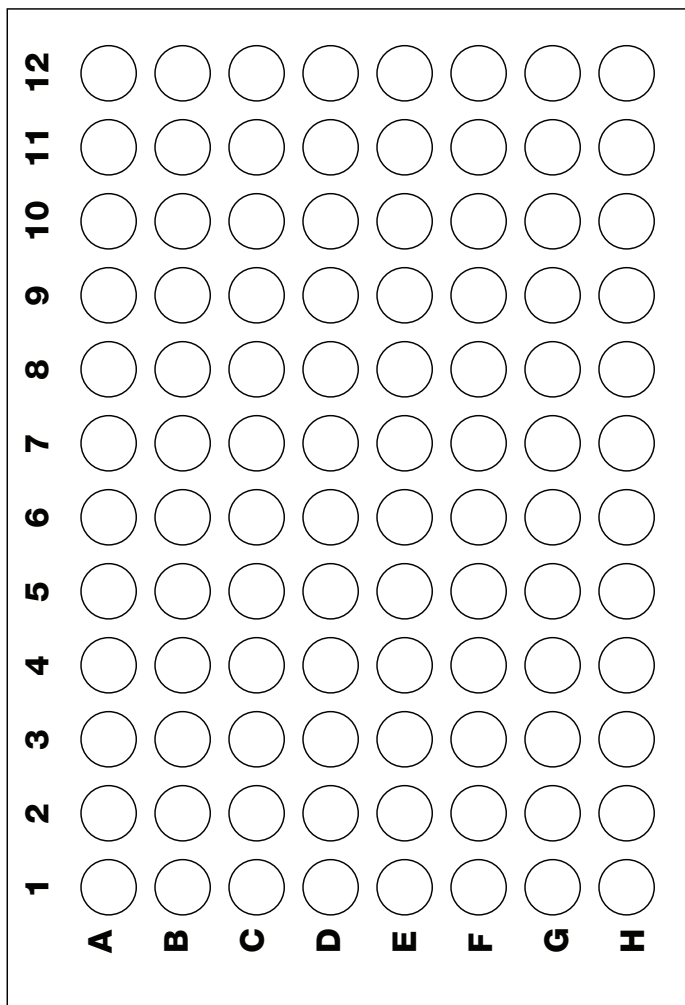


Plate Layout Template



Calculation Worksheet

Plan Plate Layout

1. Fill out the 96-well plate template as instructed in the Plan Plate Layout section.

If using either the **Diabetes fixed panel (either human or mouse) or One Diabetes single set tube/analyte**, follow these directions:

Enter the number of wells that will be used in the assay: _____ (1)

Calculations for Coupled Beads

1. Determine the volume of 1x coupled beads needed.

a) Each well requires 50 μl of coupled beads (1x): _____ (1) \times 50 μl = _____ μl (2)

b) Include a 20% excess to ensure enough volume: _____ μl (2) \times 0.20 = _____ μl (3)

c) Total volume of 1x coupled beads: _____ μl (2) + _____ μl (3) = _____ μl (4)

d) Volume of **20x coupled beads** stock: _____ μl (4)/20 = _____ μl (5)

e) Volume of **Assay Buffer** required: _____ μl (4) _____ μl (5) = _____ (6)

Calculations for Detection Antibodies

2. Determine the volume of 1x detection antibody needed.

a) Each well requires 25 μl of detection antibodies (1x): _____ (1) \times 25 μl = _____ μl (7)

b) Include a 25% excess to ensure enough volume: _____ μl (7) \times 0.25 = _____ μl (8)

c) Total volume of 1x detection antibodies: _____ μl (7) + _____ μl (8) = _____ μl (9)

d) Volume of **20x Detection Antibodies** stock: _____ μl (9)/20 = _____ μl (10)

e) Volume of **Detection Antibody Diluent** required: _____ μl (9) - _____ μl (10) = _____ μl (11)

Calculations for Streptavidin-PE

3. Determine the volume of 1x streptavidin PE needed.

a) Each well requires 50 μl of streptavidin PE (1x): _____ (1) \times 50 μl = _____ μl (10)

b) Include a 25% excess to ensure enough volume: _____ μl (10) \times 0.25 = _____ μl (11)

c) Total volume of 1x detection antibodies: _____ μl (10) + _____ μl (11) = _____ μl (12)

d) Volume of **100x Streptavidin PE** required: _____ μl (12) / 100 = _____ μl (13)

e) Volume of **Assay Buffer** required: _____ μl (12) _____ μl (13) = _____ μl (14)

If **multiplexing single set (singleplex) diabetes analytes**, follow these directions:

Enter the number of wells that will be used in the assay: _____ (1)

Calculations for Coupled Beads

1. Determine the volume of 1x coupled beads needed.

a) Each well requires 50 μl of coupled beads (1x): _____ (1) \times 50 μl = _____ μl (2)

b) Include a 20% excess to ensure enough volume: _____ μl (2) \times 0.20 = _____ μl (3)

c) Total volume of 1x coupled beads: _____ μl (2) + _____ μl (3) = _____ μl (4)

d) Enter the number of diabetes single set (or analytes) tubes that will be multiplexed = _____(5)

e) Volume of **20x Coupled Beads** required from **each diabetes coupled beads tube**:

_____ μl (4) /20 = _____ μl (6)

f) Total volume of diabetes bead stock required: _____(5) \times _____ μl (6) = _____ μl (7)

g) Volume of **Assay Buffer** required: _____ μl (4) _____ μl (7) = _____ μl (8)

Calculations for Detection Antibodies

2. Determine the volume of 1x detection antibody needed.

a) Each well requires 25 μl of detection antibodies (1x): _____ (1) \times 25 μl = _____ μl (9)

b) Include a 25% excess to ensure enough volume: _____ μl (9) \times 0.25 = _____ μl (10)

c) Total volume of 1x detection antibodies: _____ μl (9) + _____ μl (10) = _____ μl (11)

d) Enter the number of diabetes single set (or analytes) tubes that will be multiplexed = _____(5)

e) Volume of **20x Detection Antibodies** required from **each diabetes detection antibody tube**:

_____ μl (11) /20 = _____ μl (12)

f) Total volume of diabetes detection antibody stock: _____ μl (12) \times _____(5) = _____ μl (13)

g) Volume of **Detection Antibody Diluent** required: _____ μl (11) – _____ μl (13) = _____ μl (14)

Calculations for Streptavidin-PE

3. Determine the volume of 1x streptavidin PE needed.

a) Each well requires 50 μl of streptavidin PE (1x): _____ (1) \times 50 μl = _____ μl (15)

b) Include a 25% excess to ensure enough volume: _____ μl (15) \times 0.25 = _____ μl (16)

c) Total volume of 1x detection antibodies: _____ μl (15) + _____ μl (16) = _____ μl (17)

d) Volume of **100x Streptavidin PE** required: _____ μl (17) / 100 = _____ μl (18)

e) Volume of **Assay Buffer** required: _____ μl (17) _____ μl (18) = _____ μl (19)

If **multiplexing diabetes (20x) and cytokine (10x) assays**, follow these directions:

Enter the number of wells that will be used in the assay: _____ (1)

Enter the number of diabetes tubes (either single set or multiplex) that will be multiplexed: _____ (2)

Enter the number of cytokine tubes (either single set or multiplex) that will be multiplexed: _____ (3)

Calculations for Coupled Beads

1. Determine the volume of 1x diabetes and cytokines coupled beads needed.

a) Each well requires 50 μl of coupled beads (1x): _____ (1) \times 50 μl = _____ μl (4)

b) Include a 20% excess to ensure enough volume: _____ μl (4) \times 0.20 = _____ μl (5)

c) Total volume of 1x coupled beads: _____ μl (4) + _____ μl (5) = _____ μl (6)

d) **Volume of 20x diabetes coupled beads stock** required from each diabetes tube(s):

_____ μl (6) / 20 = _____ μl (7)

e) **Volume of 10x cytokines coupled beads stock** required from each cytokines tube(s):

_____ μl (6) / 10 = _____ μl (8)

f) Total volume of diabetes bead stock required: _____ μl (7) \times _____ (2) = _____ μl (9)

g) Total volume of cytokine bead stock required: _____ μl (8) \times _____ (3) = _____ μl (10)

h) Total volume of diabetes and cytokine bead stock required: _____ μl (9) + _____ (10) = _____ μl (11)

i) Volume of **Assay Buffer** required: _____ μl (6) _____ μl (11) = _____ μl (12)

Calculations for Detection Antibodies

2. Determine the volume of 1x diabetes and cytokines detection antibodies needed.

a) Each well requires 25 μl of detection antibodies (1x): _____ (1) \times 25 μl = _____ μl (13)

b) Include a 25% excess to ensure enough volume: _____ μl (13) \times 0.25 = _____ μl (14)

c) Total volume of 1x detection antibodies: _____ μl (13) + _____ μl (14) = _____ μl (15)

d) **Volume of 20x Detection Antibodies** required from each diabetes tube(s):

_____ μl (15) / 20 = _____ μl (16)

e) **Volume of 10x Detection Antibodies** required from each cytokines tube(s):

$$\text{_____ } \mu\text{l (15)} / 10 = \text{_____ } \mu\text{l (17)}$$

f) Total volume of diabetes detection antibodies stock required: _____ μl (16) x _____ (2) = _____ μl (18)

g) Total volume of cytokine detection antibodies stock required: _____ μl (17) x _____ (3) = _____ μl (19)

h) Total volume of diabetes and cytokine detection antibodies required:

$$\text{_____ } \mu\text{l (18)} + \text{_____ (19)} = \text{_____ } \mu\text{l (20)}$$

i) Volume of **Detection Antibody Diluent** required: _____ μl (15) – _____ μl (20) = _____ μl (21)

Calculations for Streptavidin-PE

3. Determine the volume of 1x streptavidin PE needed.

d) Each well requires 50 μl of streptavidin PE (1x): _____ (1) x 50 μl = _____ μl (15)

e) Include a 25% excess to ensure enough volume: _____ μl (15) x 0.25 = _____ μl (16)

f) Total volume of 1x detection antibodies: _____ μl (15) + _____ μl (16) = _____ μl (17)

d) Volume of **100x Streptavidin PE** required: _____ μl (17) / 100 = _____ μl (18)

e) Volume of **Assay Buffer** required: _____ μl (17) _____ μl (18) = _____ μl (19)