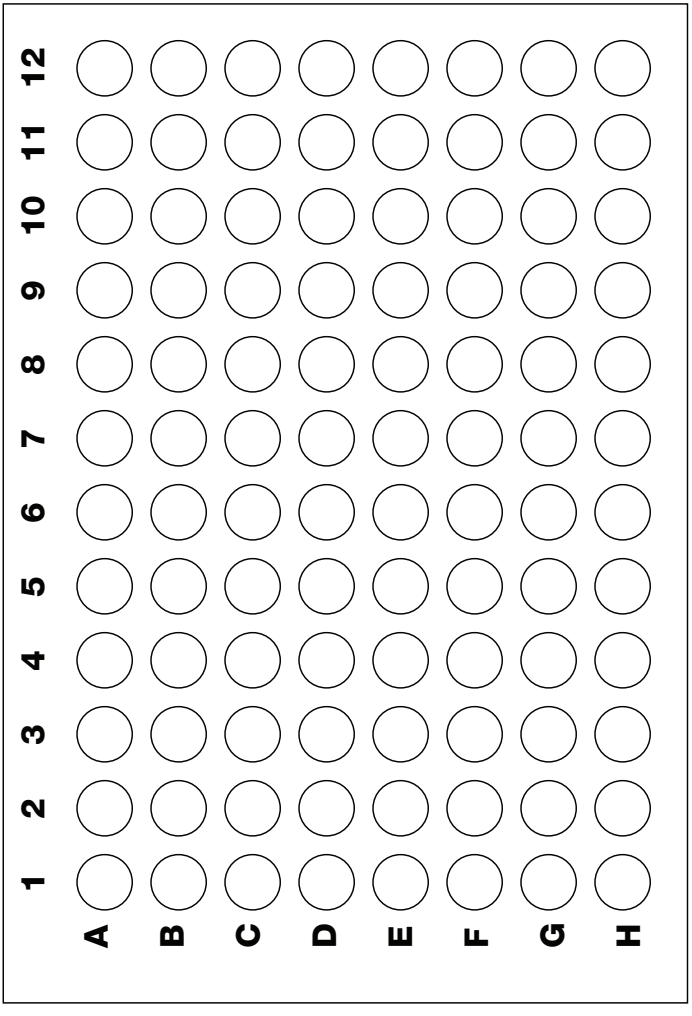
Plate Layout Template



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) x 50 µl = _____ µl (2)

   b) Include a 20% excess to ensure enough volume: _____ µl (2) x 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) x 25 µl = _____ µl (7)

   b) Include a 25% excess to ensure enough volume: _____ µl (7) x 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) x 50 µl = _____ µl (10)

   b) Include a 25% excess to ensure enough volume: _____ µl (10) x 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) x 50 µl = _____ µl (2)

   b) Include a 20% excess to ensure enough volume: _____ µl (2) x 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) x _____ 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) x 25 µl = _____ µl (9)

   b) Include a 25% excess to ensure enough volume: _____ µl (9) x 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) x _____(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) x 50 µl = _____ µl (15)

   b) Include a 25% excess to ensure enough volume: _____ µl (15) x 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) x 50 µl = _____ µl (4)

   b) Include a 20% excess to ensure enough volume: _____ µl (4) x 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) x _____ (2) = _____ µl (9)

   g) Total volume of cytokine bead stock required: _____ µl (8) x _____ (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) x 25 µl = _____ µl (13)

   b) Include a 25% excess to ensure enough volume: _____ µl (13) x 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):

_____ µl (15) / 10 = _____ µl (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:

_____ µl (18) + _____ (19) = _____ µl (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)