Plate Layout Template
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 = _______

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 = _______

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):

\[ \text{______ µl (15) / 10 = _____ µl (17)} \]

f) Total volume of diabetes detection antibodies stock required:

\[ \text{_____ µl (16) x _____ (2) =______ µl (18)} \]

g) Total volume of cytokine detection antibodies stock required:

\[ \text{_____ µl (17) x _____ (3) =______ µl (19)} \]

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

\[ \text{_____ µl (18) + _____ (19) = _____ µl (20)} \]

i) Volume of **Detection Antibody Diluent** required:

\[ \text{_____ µl (15) - _____ µl (20) = _____ µl (21)} \]

**Calculations for Streptavidin-PE**

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

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

\[ \text{_________ (1) x 50 µl = __________ µl (15)} \]

e) Include a 25% excess to ensure enough volume:

\[ \text{_________ µl (15) x 0.25 = ________ µl (16)} \]

f) Total volume of 1x detection antibodies:

\[ \text{_________ µl (15) + _____ µl (16) = _____ µl (17)} \]

d) Volume of **100x Streptavidin PE** required:

\[ \text{_________ µl (17) / 100 = _____ µl (18)} \]

e) Volume of **Assay Buffer** required:

\[ \text{______ µl (17) _____ µl (18) = __________ µl (19)} \]