# QuickReferenceCard

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## Hybridization Cocktail Preparation Instructions for Processing Whole Transcript Array Plates using the GeneTitan® Instrument and the WT Assay

**NOTE:** The "WT Hyb Add" reagent names were created to match the order in which reagents are added. For example, WT Hyb Add 4 is the fourth component added during preparation of the Hybridization Mix. WT Hyb Add 2, 3 and 5 are not used and are not part of the Hybridization Modules.

1. Remove the vials labeled **5X WT Hyb Add 1**, **15X WT Hyb Add 4** and **2.5X WT Hyb Add 6** from the GeneChip® Hybridization Module for WT Array Plates, P/N 901621.
   - **A.** Warm reagents to room temperature on the bench.
   - **B.** Vortex **5X WT Hyb Add 1**, **15X WT Hyb Add 4** and **2.5X WT Hyb Add 6** to mix. Centrifuge briefly (~5 sec) to collect liquid at the bottom of the tube.
2. Remove the GeneChip® Hybridization Control Kit from –20°C freezer and thaw at room temperature.
   - **A.** Vortex and centrifuge briefly (~5 sec) to collect liquid at the bottom of the tube.
   - **B.** Keep on ice.
3. Prepare the WT Hybridization Mix in the order as shown in Table 1. The **5X WT Hyb Add 1** solution is very viscous, pipet slowly to ensure addition of the correct volume. Mix well.

Table 1

| Order to Add Reagents | Component | Volume per Array | 16-Array Plate* | 24-Array Plate* | 96-Array Plate* | Final Concentration |
|---|---|---|---|---|---|---|
| 1 | 5X WT Hyb Add 1 | 24 µL | 422.4 µL | 633.6 µL | 2534.4 µL | 1X |
| 2 | Control Oligonucleotide B2 (3 nM) | 1.2 µL | 21.1 µL | 31.7 µL | 126.7 µL | 30 pM |
| 3 | 20X Eukaryotic Hybridization Controls (*bioB, bioC, bioD, cre*) | 6 µL | 105.6 µL | 158.4 µL | 633.6 µL | 1.5, 5, 25 and 100 pM, respectively |
| 4 | 15X WT Hyb Add 4 | 8 µL | 140.8 µL | 211.2 µL | 844.8 µL | 1X |
| **Total Volume** | | **39.2 µL** | **689.9 µL** | **1,034.9 µL** | **4139.5 µL** | |

**Includes ~10% overage to cover pipetting error.*

- **A.** Aliquot 39.2 µL of the master mix prepared in Table 1 to each tube or well. Add the fragmented and labeled single-stranded DNA target generated from the GeneChip® WT PLUS or GeneChip® WT Pico Kits.

Table 2

| Order to Add Reagents | Component | Volume per Array | Final Concentration |
|---|---|---|---|
| 5 | Fragmented and Labeled DNA | 32.8 µL | ~25 ng/µL |
| **Total Volume** | | **72 µL** | |

- **B.** Add the **2.5X WT Hyb Add 6** from the GeneTitan Hybridization Module for WT Array Plates as shown in Table 3.

Table 3

| Order to Add Reagents | Component | Volume per Array | Final Concentration |
|---|---|---|---|
| 6 | 2.5X WT Hyb Add 6 | 48 µL | 1X |
| Total Volume | | 120 µL | |

- **C.** If you are using a plate; seal, vortex, and centrifuge briefly (~5 sec) to collect liquid at the bottom of the tube. If you are using 1.5 mL tubes; vortex and centrifuge briefly (~5 sec) to collect liquid at the bottom of the tube.

4. Denature the hybridization cocktail with target at 99°C (1.5 mL tubes) or 95°C (thermocycler plates) for 5 minutes, followed by 45°C for 5 minutes.
5. After denaturation, spin hybridization cocktail with target in a centrifuge to remove any insoluble material from the hybridization mixture. If you are using 1.5 mL tubes, use the Eppendorf 5417C centrifuge (or similar). If you are using thermocycler plates, use the Eppendorf 5804R centrifuge (or similar). Spin either tubes or plates for 1 minute at 5000 RPM at room temperature.
6. Place 90 µL of the centrifuged supernatant master mix into the appropriate well of the hybridization tray.
7. Refer to the *GeneTitan® Instrument User Guide for Expression Array Plates* (P/N 702933) for details on the GeneTitan hybridization setup.

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