

GeneTitan® Hybridization, Wash, and Stain Kit for WT Array Plates

96 Reactions

Intended Use

This package insert provides information for the GeneTitan® Hybridization, Wash, and Stain Kit for WT Array Plates (P/N 901622). The kit is composed of two boxes:

- P/N 901621: GeneTitan® Hybridization Module for WT Array Plates* – sufficient for 12x8 reactions, 6x16 reactions, 4x24 reactions, or 1x96 reactions
- P/N 901583: GeneTitan® Wash Buffers A and B Module – sufficient for 1 array plate.

The GeneTitan Hybridization, Wash, and Stain Kit for WT Array Plates includes all the necessary reagents for the hybridization, wash, and stain steps of the Ambion WT Expression Kit and GeneChip WT Terminal Labeling and Controls Kit using Affymetrix® Whole Transcript (WT) Array Plates and Array Strips.

*The GeneTitan Hybridization, Wash, and Stain Kit for WT Array Plates (P/N 901622) consists of P/N 901621 and 901583. Part Number 901621 is not available for purchase separately.

Module Components

Component	Volume	Storage
GeneTitan® Hybridization Module for WT Array Plates (P/N 901621)		
5X WT Hyb Add 1	4 x 925 µL	2°C to 8°C
15X WT Hyb Add 4	4 x 355 µL	2°C to 8°C
2.5X WT Hyb Add 6	4 x 1,525 µL	2°C to 8°C
Stain Cocktail 1 & 3	22 mL	2°C to 8°C
Stain Cocktail 2	13 mL	2°C to 8°C
Array Holding Buffer	110 mL	2°C to 8°C
GeneTitan® Wash Buffers A & B Module (P/N 901583)		
Wash Buffer A	1,045 mL	2°C to 8°C
Wash Buffer B	450 mL	2°C to 8°C

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.

Shipping Information

The two components of the The GeneTitan Hybridization, Wash, and Stain Kit are shipped at different temperatures. The GeneTitan® Hybridization Module for WT Array Plates (P/N 901621) will be sent at 5°C. The GeneTitan™ Wash Buffers A & B Module (P/N 901583) will be sent at ambient temperature.

Safety Information

Warning! P/N 901621 contains Tetramethylammonium chloride (TMACl) and formamide, both of which are considered highly toxic. P/N 901621 contains Sodium Azide. May react with lead and copper plumbing to form highly explosive metal azides. A Material Safety Data Sheet(s) (MSDS) is available at www.affymetrix.com. If the product is a kit or is supplied with more than one material, please refer to the MSDS for each component for hazard information.

Functional Testing

Each lot of reagents is rigorously tested for performance.

Ordering Information

P/N	Product Name	Description/Size
901622	GeneTitan® Hybridization, Wash, and Stain Kit for WT Array Plates ¹	96 Rxn
Related Products		
901583	GeneTitan® Wash Buffers A and B Module	Sufficient for processing 1 array plate.
901647 or 901648	GeneChip® HT WT Terminal Labeling and Control Kit ² or <ul style="list-style-type: none"> ■ GeneChip® HT WT Terminal Labeling Kit (P/N 901555, 24 Rxn or P/N 901518, 96 Rxn) ■ GeneChip® Hybridization Control Kit (P/N 900454, 30 Rxn or P/N 900457, 150 Rxn) ■ GeneChip® Poly-A RNA Control Kit (P/N 900433, 100 Rxn) 	24 Rxn (P/N 901647) or 96 Rxn (P/N 901648)
901525 or 901524	GeneChip® WT Terminal Labeling and Control Kit ² or <ul style="list-style-type: none"> ■ GeneChip® WT Terminal Labeling Kit (P/N 901670, 10 Rxn or P/N 901671, 30 Rxn) ■ GeneChip® Hybridization Control Kit (P/N 900454, 30 Rxn) ■ GeneChip® Poly-A RNA Control Kit (P/N 900433, 100 Rxn) 	10 Rxn (P/N 901525) or 30 Rxn (P/N 901524)

1. Contains the GeneTitan® Hybridization Module for WT Array Plates (P/N 901621) and the GeneTitan® Wash Buffers A and B Module (P/N 901583). Part number 901583 is available for purchase separately.

2. Individual components available for purchase separately.

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Hybridization Cocktail Preparation Instructions for Processing Gene 1.1 ST Array Plates using the GeneTitan® Instrument and the WT Assay

The Hybridization Mix described in this Quick Reference Guide was specifically formulated for use with the Ambion® WT Expression Kit and the GeneChip® WT Terminal Labeling and Controls Kit.

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. **WT Hybridization Mix:** A new hybridization mix has been formulated for the Ambion WT Expression Kit and the GeneChip® WT Terminal Labeling and Controls Kit.
 - A. 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 (<i>bioB</i> , <i>bioC</i> , <i>bioD</i> , <i>cre</i>)	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.

- B. 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 Terminal Labeling and Hybridization User Manual for use with the Ambion® WT Expression Kit* (P/N 702808) as shown in Table 2.

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	

- C. 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	

- D. 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 *GeneChip WT Terminal Labeling and Hybridization User Manual for use with the Ambion WT Expression Kit* (P/N 702808) for details on the GeneTitan hybridization setup.