

Quick Reference Card



Hybridization Cocktail Preparation Instructions for Processing HT Array Plates using the GeneTitan™ Instrument

Hybridization Cocktail Preparation Instructions

Introduction

This QRC is for users who are preparing the hybridization cocktail mix using the HT Hybridization, Wash, and Stain Kit for the HT array plates on the GeneTitan™ Instrument.

IMPORTANT: This quick reference card is for use with the **One-Cycle Target Labeling and Control Reagents** only. It **cannot** be used for the 3' IVT Express reagents.

Reagents and Materials Master List for Hybridization, Wash and Stain

The following is a comprehensive list of the reagents and materials required for preparing the hybridization cocktail. Each section in this quick reference card will define the specific reagents and materials used in that section.

Affymetrix has tested and evaluated all reagents and materials listed. The Affymetrix United States catalog contains the relevant information and part numbers. The reagents and materials required for preparing the hybridization cocktail are as follows:

- **GeneChip® HT Hybridization, Wash, and Stain Kit (P/N 901219) includes:**

- **Box 1 of 2:**

- » HT Pre-Hybridization Mix (Not required for preparing plates for processing on the GeneTitan Instrument)
- » HT 1.2X Hybridization Mix
- » HT Stain Cocktail 1 & 3
- » HT Stain Cocktail 2
- » HT Array Holding Buffer

- **Box 2 of 2:**

- » HT Wash Buffer A
- » HT Wash Buffer B

- GeneChip Wash Buffer B (P/N 900722)
- De-ionized water
- Anti-static gun (Affymetrix PN 74-0014)

- GeneChip HT Array Holding Buffer—P/N 901218)¹
- HT Hybridization Tray—Affymetrix, P/N 500867, labeled as HT Hybridization Tray)²
- HT Scan Tray—Affymetrix P/N 900746 (kit), labeled as HT Scan Tray P/N 500860²
- GeneTitan Stain Tray—Affymetrix P/N 501025 (qty 3)²
- GeneTitan Stain and Scan Tray Covers — Affymetrix P/N 202757 (qty 4)²
- 96-Well Hard-Shell PCR Plate—Bio-Rad, P/N HSP-9601 (qty 1)



Figure 1. Components of the GeneChip® HT Hybridization Wash and Stain Kit

¹ The Array Holding buffer is included with the HT Hyb Wash Stain Kit and is also available for purchase separately.

² HT Hybridization, Stain and Scan trays along with the covers are packaged with the HT Array Plates.

Hybridization Setup

Introduction

This section describes the GeneTitan Setup protocol for HT array plates using GeneTitan™ HT Hybridization, Wash, and Stain Kit reagents. The reagent consumption per process (on the GeneTitan™ Instrument for processing HT array plates) is shown in table 1.

	HT Hyb Wash Stain Kit for GeneTitan	Wash Buffer B	Number of plates that can be processed with combination of 901219 & 900722 kits	
			96-Format	24-Format
	901219 kit	900722	96-Format	24-Format
Reagents	Vol.	Vol.	Plates	Plates
Wash A	2L		2	2
Wash B	150 mL	600 mL	2	2
Stain 1 and 2	22 mL		1	4
Stain 3	13 mL		1	4
Array Holding Buffer	20 mL		1	1

Fluid Type	Amount required in the bottle to process one HT array plate	Amount required in the bottle to process two HT array plates concurrently
Rinse	450 mL	750 mL
Wash A	1040 mL+	1950 mL
Wash B	450 mL	750 mL

NOTE: The amount of reagent volumes listed in Table 2 take into account a minimum overage of 150 mL required for processing plates.

NOTE: Each HT array plate processed on the GeneTitan instrument will generate approximately 1.5 L of fluid in the waste bottle.

NOTE: Refer to Table 3 for preparing Hybridization Cocktail Master Mix. The 120 µL final volume is sufficient for hybridizing one peg array. Scale this volume appropriately depending on the number of peg arrays being hybridized.

IMPORTANT: It is imperative that frozen stocks of 20X GeneChip® Eukaryotic Hybridization Controls are heated to 65 °C for 5 minutes to completely re-suspend the cRNA before aliquotting.

IMPORTANT: The target concentration of the labeled and fragmented 3'IVT target must be 0.5 µg /µL.

Hybridization Cocktail Master Mix for Cold Reagent Block using the GeneChip™ HT Hybridization, Wash and Stain Kit

Procedure

De-ionize the three stain trays, scan tray and their covers using the hand-held static gun (P/N 74-0014).

1. Complete the target preparation through the fragmentation step using the method of your choice, i.e., either manual target preparation or automated target preparation.
2. Obtain the 96-well hard-shell PCR plate for preparing the hybridization cocktail master mix.
3. Refer to Table 3 for 120 µL of the Hybridization Cocktail Master Mix composition. The volumes are given for 1 well array. Combine the components as indicated in each well of the 96-well hard-shell PCR plate.

IMPORTANT: Add the sample directly to the bottom of each well of the PCR plate. Thoroughly pipette-mix the contents of each well.

Reagent	Volume per Rxn
20X Hybridization Control Stock	6 µL
3 nM B2 Oligo	2 µL
HT 1.2X Hybridization Mix *	100 µL
Labeled and Fragmented 3'IVT Target.(6 µg or 0.5ug per µL)	12 µL
Total Volume	120 µL

* The 1.2X Hybridization Mix is a component of the HT Hybridization, Wash, and Stain Kit containing BSA, HS DNA and hybridization buffer.

4. Denature the hybridization-ready target for 5 minutes at 95° C and cool it down to 45 °C for 5 minutes.
5. After denaturation spin hyb target in a centrifuge to collect any insoluble material from the hybridization mixture. If you are using **1.5 mL tubes, use the Eppendorf 5417C** centrifuge (or similar centrifuge). If you are using **thermocycler plates, use the Eppendorf 5804R** centrifuge (or similar centrifuge). Spin either tubes or plates for 5 minutes at 5000 RPM at room temperature.
6. Aliquot the 90 µL of the centrifuged supernatant master mix as indicated into the appropriate well of the hybridization tray identified with the label "HT Hybridization Tray P/N 500867" on the tray.

IMPORTANT: Do not touch the bottom of the tube or well or disturb the pellet that may have formed after centrifugation.

WARNING: The de-ionization steps 7 and 8 will damage the HT arrays on the plate. Before using the anti-static gun, ensure that the HT array plates remain in their protective pouch and placed away from the de-ionization area.

7. Place all the stain trays required to process a run on a table top. Use the anti-static gun and aim at the center of each 16-well cluster and pull the trigger so that the stream of ionized particles settles on all wells of the stain tray and dissipates the static electricity.

- Place the stain tray cover with the flat surface facing upward on the table top. Aim the anti-static gun (P/N 74-0014) approximately one-half inch away from the flat surface and pull the trigger, while moving the gun across the cover, so that the stream of ionized particles settles on all areas of the cover and dissipates the static electricity from the cover.

IMPORTANT: For steps 9-15, aliquot the reagents carefully to prevent bubbles from forming. If bubbles should form remove them using a pipette tip.

- Aliquot 105 μ L of the Stain 1 into the GeneTitan Stain Tray identified with the label “GeneTitan™ Stain Tray P/N 501025” on the tray.

- After dissipating the static electricity, use the stain cover provided with the GeneTitan Consumable Upgrade kit to cover the Stain 1 tray.

IMPORTANT: You must dissipate the static electricity from every cover before placing on top of the stain trays.

- Aliquot 105 μ L of the Stain 2 into the GeneTitan Stain Tray identified with the label “GeneTitan™ Stain Tray P/N 501025” on the tray.

- Use the stain tray cover provided with the GeneTitan Consumable Upgrade kit to cover the Stain 2 tray.

- Aliquot 105 μ L of the Stain 3 into the GeneTitan Stain Tray identified with the label “GeneTitan™ Stain Tray P/N 501025” on the tray.

- Use the stain tray cover provided with the GeneTitan Consumable Upgrade kit to cover the Stain 3 tray.

- Aliquot 150 μ L of the Array Holding Buffer into the GeneTitan Scan Tray identified with the label “HT Scan Tray P/N 500860” on the tray.

IMPORTANT: You must fill all 96 wells with the Array Holding Buffer whether you use 24-well or 96-well format HT array plates.

- Use the scan tray cover provided with the GeneTitan Consumable Upgrade kit to cover the scan tray.

IMPORTANT: The instrument must have a minimum amount of reagent buffers for each HT array plate prior to starting Hybridization-Wash/Stain-Scan process. Refer to Table 2 for minimum reagent volumes required in the reagent bottles

- Load all the consumables including the HT Array Plate into the GeneTitan™ Instrument. Refer to the Affymetrix GeneChip Command Console 2.0 User Guide (P/N 702569).

IMPORTANT: It is important not to bump the trays while loading the trays into the GeneTitan instrument – Droplets of the stain on the lid may result in wicking action with the result that the instrument gripper may be unable to remove the lids properly.

Master List - Consumables, Reagents, and Equipment

You will need the following reagents and supplies to complete the hybridization cocktail preparation for processing HT array plates on the GeneTitan™ Instrument. The reagent quantities listed are for one plate of 96 well reactions.

IMPORTANT: All laboratory ware, including pipettes, must be RNase/DNase-free.

Instruments

Instruments	Supplier	P/N	Quantity / Run
GeneTitan™ Instrument	Affymetrix	00-0360	1
12-multichannel 20 μ L Pipetman	Rainin	L12-20	1
12-multichannel 200 μ L Pipetman	Rainin	L12-200	1
8-multichannel 20 μ L Pipetman	Rainin	L8-20	1
8-multichannel 200 μ L Pipetman	Rainin	L8-200	1

Consumables – Hybridization – Wash/Stain-Scan Setup

Item	Source	P/N	Quantity
HT Hybridization Tray	Affymetrix	900747	1
GeneTitan Stain Tray	Affymetrix	901334	3
HT Scan Tray	Affymetrix	900746	1
GeneTitan Stain and Scan Tray Covers	Affymetrix	202757	4
96-Well Hard-Shell PCR Plate	Bio-Rad	HSP-9601	1
Anti-Static Gun	Affymetrix	74-0014	1

Reagents –Hybridization, Wash and Stain and Scan

Material	Source	P/N
• GeneChip® HT Hybridization, Wash, and Stain Kit		901219
• GeneChip® HT Wash Buffer B		900722
Affymetrix® Eukaryotic Hybridization Control Kit containing:	Affymetrix	900454 (30 Rxns) 900457 (150 Rxns)
• GeneChip® Control Oligo B2		
• 20X Hybridization Controls		

Affymetrix Contact Information

When to Contact Technical Support

Under any of the following conditions, unplug the instrument from the power source and contact Affymetrix Technical Support:

- when the power cord is damaged or frayed;
- if any liquid has penetrated the instrument;

If the instrument must be returned for repair, call Affymetrix Technical Support.

IMPORTANT: Make sure you have the model and serial number.

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