Accuris[™] Hot Start Tag Master Mix and Hot Start Master Mix Red **General Guidelines** Description

Accuris Hot Start Tag Master Mix and Hot Start Master Mix Red are single tube formulations containing antibody mediated Accuris Hot Start Tag in a buffer engineered for fast cycling with higher reproducibility and better efficiency. After PCR, samples amplified with Master Mix Red can be loaded directly onto an agarose gel without the addition of a loading buffer - the included dye is sufficiently dense to sink to the bottom of the wells. The red dye migrates with 800-1000bp DNA fragments and the yellow dye migrates with 20-30bp DNA fragments in a 1% agarose gel.

-Ready to use mix reduces pipetting steps and errors

- -Performs across a wide range of DNA templates including genomic DNA and GC-rich and AT-rich sequences
- -Proprietary buffer system includes enhancers for maximizing enzyme activity and reaction speed
- -Improved solubility and template affinity

Storage

Upon receipt, immediately store at -20°C. Avoid excessive freeze/thaw cycles. When stored as directed, this product will retain its activity for 12 months from date of receipt. May be stored at 4°C for up to one month.

Limitations of Use

For research purposes only. Not intended for therapeutic or diagnostic use.

Quality Control

Accuris enzymes and reagents are tested under general assay conditions for activity, reproducibility, efficiency, heat activation, sensitivity, and absence of nuclease contamination and nuclease activity. This product is manufactured under a comprehensive quality management system, following ISO 9001:2008 standards.

1. 2X Tag Master Mix/Master Mix Red The Master Mix contains Accuris Tag DNA polymerase, 2mM dNTPs, 6mM MgCl., and proprietary PCR enhancers. Master Mix Red also contains an inert loading dye Formulated for maximum efficiency, sensitivity and successful PCR with a variety of difficult templates, adding additional PCR enhancers may have a negative effect.

2. Template

For PCR of complex genomic DNA, 5ng -500ng of template DNA may be added per reaction. Do not add more than 100ng of cDNA or plasmid DNA.

3. Primers

Primers should have a predicted melting temperature of approximately 60°C, using default Primer 3 settings (http://frodo.wi.mit. edu/primer3). The final primer concentration should be 0.2µM to 0.6µM.

4. Annealing Temperature

Perform gradient PCR or start at 55°C and increase in 2°C increments to find the optimal annealing temp. Proprietary enhancers in the master mix may reduce the optimal annealing temperature compared to traditional PCR buffers and mater mixes.

5. Extension

The polymerase performs optimally at 72°C. Extension time is dependent upon amplicon complexity and length. Generally, 15 seconds per kb is recommended for eukarvotic genomic DNA and cDNA. A one second extension is sufficient for shorter amplicons.

Technical Support

For trouble-shooting and tech support, contact us at info@accuris-usa.com or call 908 769-5555.

Accuris is not responsible for consequential or incidental damages, direct or indirect, resulting from use of this product. Accuris guarantees the performance of this product as described when used in accordance with these instructions.

Reaction setup

Allow the Master Mix to thaw. Thoroughly mix contents by gently pipetting up and down. Prepare the reaction as follows:

Component	25 μl reaction	50 μl reaction	Final concentration
Accuris 2X Hot Start Taq Master Mix Red	d 12.5 μl	25 µl	1X
Forward Primer (10µM)	1.0 μl	2.0 μl	400 nM
Reverse Primer (10µM)	1.0 μl	2.0 μl	400 nM
Template DNA	<100ng cDNA, ·	<500ng genomic	variable
PCR-grade water	to final reaction volume		

For other volumes, adjust the amount of each component accordingly.

Gently mix the solution. If needed, spin briefly in a microcentrifuge to bring reaction mixture to the bottom of the tube. Transfer samples to a thermal cycler and begin cycling. Be sure to include an initial activation/denaturation step of 1-2 minutes.

Routine PCR Cycling

Step	Temperature	Time	
Enzyme activation	95°C	1-2 minutes	
	95°C	15 seconds	
55-40 cycles	55°C to 67°C*	15 seconds	
	72°C	15-30 seconds per Kb	

*Annealing temperature determined by the user. See "General Guidelines".

Accuris offers a full line of PCR enzymes and master mixes. Visit www.accuris-usa.com for details.

Package contents and reordering

Accuris Hot Start Tag Master Mix and Master Mix Red are supplied in 200 and 1000 reaction (50 µl) packages.

Accuris Hot Start Tag Master Mix, Sample Catalog nos: PR1001-HS-S

PR1001-HSR-S (w/red dye) Includes 125 µl of 2X Master Mix (Master Mix Red contains red loading dye) (5 reactions).

Accuris Hot Start Tag Master Mix, 200 Rxns Catalog nos: PR1001-HS-200

PR1001-HSR-200 (w/red dye) Includes 5ml of 2X Master Mix (Master Mix Red contains red loading dye) in 1.25ml aliguots.

Accuris Hot Start Tag Master Mix, 1000 Rxns Catalog nos: PR1001-HS-1000

PR1001-HSR-1000 (w/red dye) Includes 25ml of 2X Master Mix (Master Mix Red contains red loading dye) in 1.25ml aliquots. 12/18





Package contains: 5ml of 2X Hot Start Tag Master Mix (4x1.25ml) 200 reactions, Based on 50µl total reaction volume Store at -20°C upon receipt PH: 908.769.5555 EM: Info@accuris-usa.com