

AttoMaster 2X Mix

Product number **AM10** **1.25 ml**

Store at –20°C. 125-20 µl reactions

Contains Taq polymerase (requires heat activation), dNTPs (0.4 mM) with optimal dUTP to dTTP ratio, heat labile UDG, Mg(6 mM), and buffer.

Typical use: Add Attostar Primer-probe, DNA, and then thermal cycle.

This table contains typical component volumes:

RotorGene Reagents needed for 20 ul PCR final reaction tube volumes										
Reaction tube number	1	2	3	4	5	6	7	8	9	10
Attostar Primer-Probe FAM labeled (10X)	2	4	6	8	10	12	14	16	18	20 µl
Master mix (2X)	10	20	30	40	50	60	70	80	90	100 µl
Dispense 12 ul / reaction tube										
Add 8 ul DNA / reaction tube										

This table includes the use of BSA, helpful for the LightCycler glass capillaries.

LightCycler Reagents needed for 20 ul PCR final reaction tube volumes										
Reaction tube number	1	2	3	4	5	6	7	8	9	10
Attostar Primer-Probe (10X)	2	4	6	8	10	12	14	16	18	20 µl
Master mix (2X)	10	20	30	40	50	60	70	80	90	100 µl
BSA 1 mg/ml	1	2	3	4	5	6	7	8	9	10 µl
Dispense 13 ul / reaction tube										
Add 7 ul DNA / reaction tube										

Other components may be added to the AttoMaster Mix, keeping the 2X AttoMaster Mix proportions.

Thermal cycle conditions for PCR reactions on RotorGene*

*Similar cycle conditions and reaction volumes may be used on many other thermal cyclers.

25°C 10 min (UDG treatment time)
95C 120 sec (activation for AttoMaster polymerase)
40 cycles
 95C 15 seconds
 60C 30 seconds RotorGene Channel Setup FAM/Sybr, Cy5; Gain 7
 72C 30 seconds

FAM/Sybr has a source of 470nm and Detector 510nm (LightCycler use F1)
Cy5 has a source of 625nm and Detector 660hp nm
Quasar 670 has the same fluorescent absorption and emission as Cy5.

.....
Thermal cycle conditions for PCR reactions on LightCycler

25°C 10 min (UDG treatment time)
95C 120 sec (activation for AttoMaster polymerase)
40 cycles
 95C 15 seconds
 60C 30 seconds acquire fluorescent signal on F1 gain =1
 72C 30 seconds
40C 30 seconds cool