xTAG® CYP2C19 Kit v3 (I046B0427)

Refer to the product package insert MLD-046-KPI-001 Rev A for complete instructions, warnings and precautions.

A. PCR

1. Thaw reagents at appropriate temperatures.
2. Label appropriate number of 0.2 mL thin-wall PCR tubes.
3. Label one 1.5 mL centrifuge tube as "MM" for Master Mix.
4. Prepare PCR Master Mix:

2C19v3 Reagent	x 1 Reaction (µL)
DNAse, RNAse-Free Distilled Water	5.75
xTAG® 10x HS Taq Polymerase Buffer	3.00
xTAG® CYP2C19 Kit v3 PCR Primer Mix	3.00
xTAG® Hot Start Taq, keep at -20°C until ready to use (flick/spin)	0.25

5. Mix/Spin PCR Master Mix.
6. Aliquot 12µL of Master Mix to each PCR tube.
7. Add 3µL of sample/control to each tube. Use dH_2O as Neg (Spin/Mix/Spin). [50ng/µL Total Conc. Optimized]
8. Run cycler (store 2-8°C for up to 24 hrs max).

B. Amplicon Treatment

1. Mix/Spin PCR tubes.
2. Prepare Amplicon Treatment Master Mix:

2C19v3 Reagent	x 1 Reaction (µL)
xTAG® Shrimp Alkaline Phosphatase, (flick/spin)	1.20
xTAG® Exonuclease I, (flick/spin)	0.30

3. Mix/Spin Master Mix.
4. Add 1.5µL of the Amplicon Treatment Master Mix to each tube containing PCR product (Spin/Mix/Spin).
5. Run cycler (store at 2-8°C for up to 4 hrs).

C. ASPE

1. Thaw reagents at appropriate temperatures.
2. Label appropriate number of 0.2 mL thin-wall PCR tubes.
3. Label one 1.5 mL centrifuge tube "ASPE MM".
4. Prepare ASPE Master Mix:

2C19v3 Reagent	x 1 Reaction (µL)
DNAse, RNAse-Free Distilled Water	7.40
xTAG® 10x HS Taq Polymerase Buffer	5.00
xTAG® CYP2C19 Kit v3 ASPE Primer Mix	4.25
xTAG® Hot Start Taq	0.35

5. Mix/Spin ASPE Master Mix.
6. Aliquot 17µL of Master Mix to each 0.2 mL thin-wall PCR tube.
7. Add 3µL treated PCR product to each tube. Mix/Spin.
8. Run cycler (store at 2-8°C for up to 24 hrs max).

D. Bead Hybridzation

1. Thaw reagents below at room temperature.
2. Cut/Label Costar plate.
3. Vortex/Sonicate Bead Mix 10 sec. Repeat.
4. Aliquot 20.0 µL of xTAG CYP2C19 Kit v3 Bead Mix to each well.
5. Transfer 1 µL of ASPE product to corresponding wells. Pipet up/down to mix.
6. Cover with Microseal.
7. Run cycler.

E. Reporter Addition

1. Prepare reporter solution (1:75 dilution):

2C19v3 Reagent	x 1 Reaction (µL)
xTAG® Reporter Buffer	74.0
xTAG® Streptavidin, R-Phycoerythrin Conjugate G75	1.00

2. Pipette 75µL of diluted reporter solution into hybridized samples.
3. Pipette up/down to mix.
4. Incubate at RT for 15 minutes in the dark.
5. Run on Luminex® 100/200™ system.

For accurate results, read the package insert and follow the instructions carefully.

For in vitro diagnostic use only.

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