VetMAX™-Plus qPCR Master Mix

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *VetMAX™-Plus qPCR Master Mix Protocol* (PN 4426032). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Procedure

1 Before you begin: isolate the DNA

Use a MagMAX™ nucleic acid isolation kit to isolate the DNA. For optimal results, Applied Biosystems recommends the following:

- Use a MagMAX nucleic acid isolation kit that is appropriate for your sample type. Go to **www.appliedbiosystems.com**, then search for **MagMAX**.
- Use pure DNA that is free of PCR inhibitors.
- Add Xeno DNA Control to the MagMAX lysis/binding solution concentrate that is used for the DNA isolation, to serve as a positive control for the recovery of DNA and for the PCR. Add 2 µL of undiluted Xeno DNA Control (20,000 copies) per isolation. For example, for ten isolations, you would:
  1. Start with enough MagMAX lysis/binding solution concentrate for ten isolations.
  2. Add 20 µL of Xeno DNA Control to the MagMAX lysis/binding solution concentrate, vortex briefly, add isopropanol, then vortex to mix.
- When isolating viral DNA from cell-free sample sources such as serum, add carrier DNA to the MagMAX lysis/binding solution concentrate to maximize DNA recovery. Include carrier DNA even if you added Xeno DNA Control to the lysis/binding solution concentrate.

2 Prepare the reactions

a. Prepare the PCR mix on ice or at room temperature (see the required volumes in the table below).
- Prepare 10% extra PCR mix.
- Include duplicate no-template controls (NTCs or negative controls) using Nuclease-free Water in place of sample.

b. Add the PCR mix to a reaction plate or tubes.

c. Add the sample to each reaction and mix well.

	Component	Volume (µL)
PCR mix	**2× qPCR Master Mix**	12.5
	PCR primer/TaqMan® probe mixture	__
	Nuclease-free Water	to 12.5
	DNA sample‡ (Nuclease-free Water for controls)	__
	Total volume per reaction	25.0

‡ Applied Biosystems recommends using <1 µg of input DNA.

3 Perform the run (PCR)

a. Program your real-time PCR instrument using the thermal-cycling conditions shown in the tables below.

- ROX™ passive reference dye is included in the 2× qPCR Master Mix.
- For real-time PCR instruments capable of Fast thermal cycling, set the mode to *Standard*.
- The reaction volume is 25 µL.

Non-MGB probe thermal cycling conditions‡

	Stage	Reps	Temp	Time
Enzyme activation/template denaturation	1	1	95 °C	10 min
Amplification	2	40	95 °C	15 sec
			60 °C	45 sec§

‡ Applied Biosystems recommends the non-MGB thermal cycling conditions for non-MGB probes, such as Eclipse® Q and Black Hole Quencher® dyes.
§ Longer templates may require an extension time >45 seconds.

MGB probe thermal cycling conditions

	Stage	Reps	Temp	Time
Enzyme activation/template denaturation	1	1	95 °C	10 min
Amplification	2	40	95 °C	15 sec
			55 °C	45 sec‡

‡ Longer templates may require an extension time >45 seconds.

b. Start the run according to the instructions for your real-time PCR instrument.

4 Analyze the data

Analyze the data according to the instructions for your real-time PCR instrument. Applied Biosystems recommends the following:

Recommendation	Details
Use the Auto C_T setting for data analysis.	This setting minimizes subjectivity when setting the threshold cycle (C_T) for the Xeno DNA Control and sample amplifications.
If the Auto C_T setting does not produce satisfactory results, manually set the thresholds that are used to determine the C_T values.	See the protocol for procedures on manually setting the thresholds.
Check the raw fluorescence data.	Verify that fluorescence increases seen in the normalized data are also evident without mathematical data processing.

Part Number 4426035 Rev. A 03/2009

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