

# Third Wave Technologies

## Universal Invader™ Reaction Guide

This document has been designed in order to assist you with the use of the Third Wave Technologies (TWT) Universal General Purpose Reagents and oligonucleotides designed by the Universal Invader™ design software when setting up reactions. These guidelines are intended to provide the user with a starting point for the development of reactions, individual reactions should be optimized to obtain the best possible performance.

**!NOTE:** For more information on the Universal Invader™ chemistry see the TWT Universal Invader™ chemistry tutorial.

**!NOTE:** For more information on designing Universal Invader™ oligonucleotides see the Universal Invader™ design software tutorial.

### General Workflow for an Universal Invader™ Reaction

1. The Universal 10X Buffer and oligonucleotides should be allowed to come to room temperature before use, keep Universal 40X Enzyme mix in freezer until just prior to use.
2. Make concentrated oligonucleotide mix. Suggested ranges of final concentrations of various reagents in the Universal Invader™ reaction are shown in **Table 1**. You may want to start at the lower concentration and work your way up to higher concentrations for optimizing your Universal Invader™ reactions.

**!NOTE:** This step may be done any time prior to beginning the Universal Invader™ reaction. It is suggested that the oligonucleotide mixture be stored at -20 to 4 degrees C.

Component	Reaction Concentration
Each Primer Oligonucleotide	0.1 $\mu$ M – 1.0 $\mu$ M
Invading Oligonucleotide	0.01 $\mu$ M – 0.1 $\mu$ M
Each Probe Oligonucleotide	0.25 $\mu$ M– 0.67 $\mu$ M
Each Universal FRET Oligonucleotide	0.125 $\mu$ M– 0.5 $\mu$ M

**Table 1.** Example final oligonucleotide concentration ranges in Universal Invader™ reaction.

3. Prepare master mix by combining the following reagents.
  - a. Universal 10X Buffer
  - b. Universal 40X Enzyme Mix
  - c. Oligonucleotide mix prepared in Step 2.
  - d. Water of suitable quality for molecular biology may be added to make the volume of reaction mix in each reaction more easily transferable.
4. Set up reaction.
  - a. Add master mix to reaction vessel.
  - b. Add desired sample to reaction vessel. Mixing upon addition of sample may improve results.

**!NOTE:** Some sample extraction methods may contain inhibitors so it may be necessary to dilute sample to minimize any effect on the Universal Invader™ reaction.

- c. Seal reaction vessel thoroughly to prevent evaporation during reaction.
5. Place reaction vessel in an incubator capable of running PCR and maintaining 99°C for 10 minutes or more. Run Universal Invader™ reaction which includes the steps below. An example cycling profile is shown in **Figure 1**. Based on the characteristics of your starting nucleic acid material you will have to modify the profiles cycle number and or incubation times to obtain the best results.
  - a. Amplification of nucleic acid by PCR.
  - b. Heat destruction of DNA Polymerase.
  - c. Invader® reaction incubation.
  - d. It is recommended to end the method with a refrigerated hold step.

Step	Step Description	Temperature (°C)	Time (Minutes)	Cycles
1	Denature Sample	95	2.0	2
2	Anneal and Extend Primers	72	2.0	
3	Denature Sample	95	0.5	22
4	Anneal and Extend Primers	72	1.0	
5	Heat Destroy DNA Polymerase	99	10.0	1
6	Invader® Reaction Incubation	63	10.0	1
7	Refrigerated Hold	10		1

**Figure 1.** Example Universal Invader™ reaction cycling profile.

6. After running the Universal Invader™ reaction collect resulting fluorescence data. The peak excitation and peak emission values for the Universal FRET dyes provided by TWT are given in **Table 2**.

	FAM	RED	YELLOW
Universal Arm1 FRET	Part #: 96-007	Part #: 96-008	Part #: 96-009
Universal Arm3 FRET	Part #: 96-010	Part #: 96-011	
Universal Arm4 FRET	Part #: 96-013	Part #: 96-014	
Universal Arm6 FRET	Part #: 96-015	Part #: 96-016	
Peak Excitation:	495 nm	580 nm	538 nm
Peak Emission:	518 nm	594 nm	559 nm

**Table 2.** Universal FRET information.

7. Analyze the data using method of choice.

**!NOTE:** There are several applications for the Universal Invader™ chemistry and when designing the reaction it should be considered how the different populations will be distinguished from each other and from the background level of fluorescence.