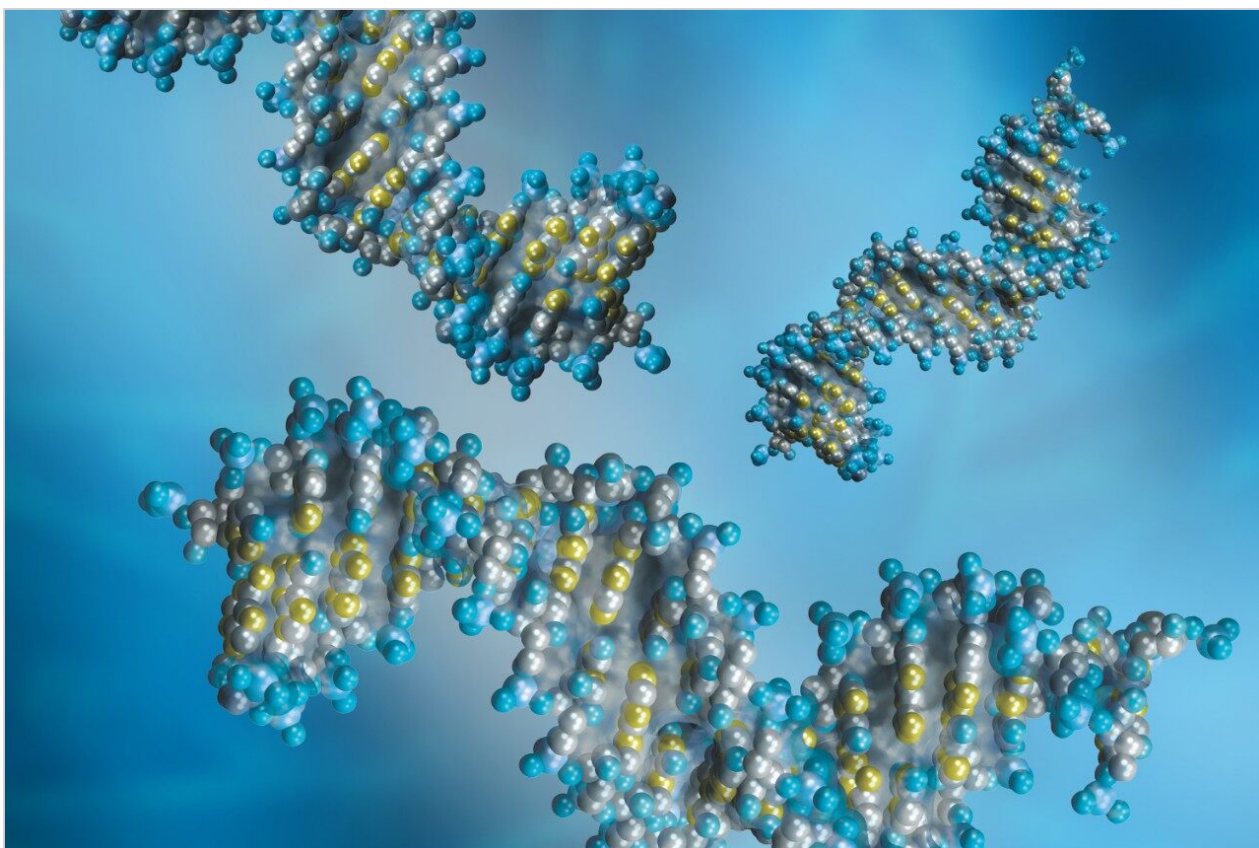


## RP-HPLC Method for Purification of Dually-Labeled Synthetic Oligonucleotides

---

Waters Corporation



---

### Abstract

This application note demonstrates RP-HPLC method for purification of dually-labeled synthetic oligonucleotides.

---

## Introduction

### Dually-Labeled Oligonucleotide Probes

Dually-labeled oligonucleotides are used mainly in quantitative polymerase chain reactions (qPCR), genotyping, and for diagnostic applications. Most common dually-labeled oligonucleotides are TaqMan probes, labeled with 5'FAM and 3' TAMRA dyes. Synthesis of these oligonucleotides is challenging, especially when a "one-pot" approach is adopted without semi-product purification. A target product is contaminated with the partially labeled, non-labeled oligonucleotides, and excess of dyes. The impurities interfere with molecular biology assays and have to be re-moved.

---

## Results and Discussion

### XTerra Column for Fast and High-Performance TaqMan Probes Purification

XTerra MS C<sub>18</sub> Columns are packed with porous 2.5 μm, hybrid particles. The sorbent has extended stability at temperatures and pH's typically used for oligonucleotide separations (50-60 °C; pH 7-9). Non-labeled oligonucleotides, singly-labeled failure sequences, and excess of free dyes can be easily removed from the target product using XTerra MS C<sub>18</sub> Columns. Figure 1 shows the purification of 100 nmole of 31mer TaqMan oligonucleotide produced in a "one-pot" synthesis. Undesirable failed products can be can be successfully removed from product using an XTerra Column. The mobile phases are volatile and can be removed from collected fractions by evaporation. The 4.6 x 50 mm column is suitable for purification of 50–200 nmole scale synthesis in a single injection. Larger amounts (200 nmole–1 μmole) of oligonucleotides can be purified using 10 x 50 mm column.

**HPLC system:** Alliance® 2695 Separations Module  
**Column:** XTerra®MS C<sub>18</sub>, 2.5 μm, 4.6 x 50 mm  
**Part Number:** 186000602  
**Flow rate:** 0.5 ml/min  
**Solvent A:** 5 % ACN in 0.1 M TEAA, pH 7  
**Solvent B:** 40 % ACN in 0.1 M TEAA, pH 7

**Gradient:** From 17.1 % B to 60% B in 30 min  
**Injection volume:** 5-50 μl (5-50 nmole of oligonucleotide)  
**Column temp.:** 60 °C  
**Detection:** UV @ 260 nm and real time UV-VIS 220-600 nm

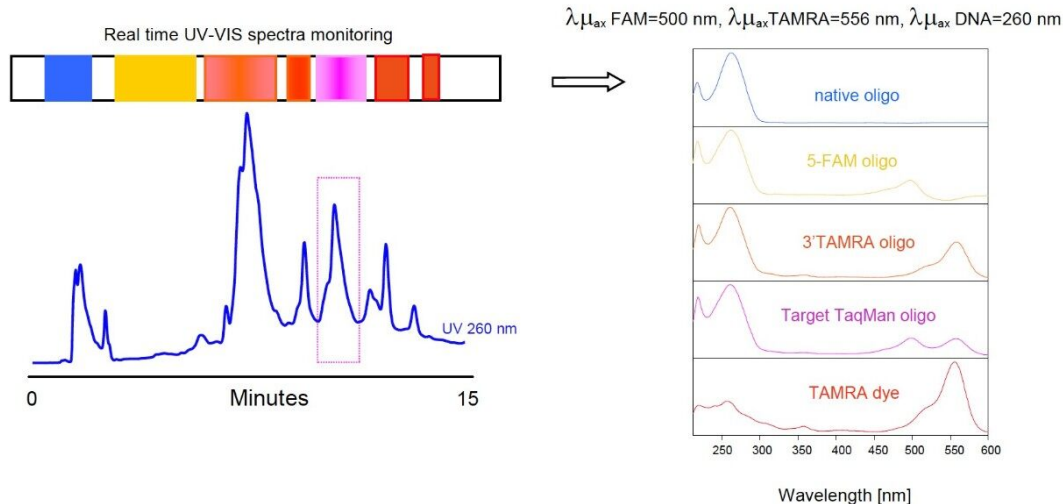
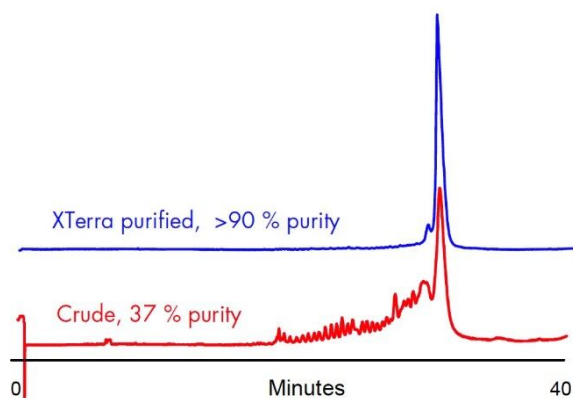


Figure 1. HPLC purification of 100 nmole of 36mer TaqMan oligonucleotide synthesized using "one-pot" approach.



**CGE system:** Beckman-Coulter P/ACE™ MDQ  
**Column:** BioRad BioCAP™ 75 μm, 34.5 cm  
**Separation:** 1.5 kV  
**Sieving matrix:** PEG 35 kDa, 2 g/10 ml 0.1 mM Tris-Boric acid, pH 8.3  
**Injection:** 4 s @ 5kV  
**Column temp.:** 30 °C  
**Detection:** UV @ 260 nm

Oligonucleotide was purified on XTerra column (Figure 1)

Figure 2. Capillary Gel Electrophoresis (CGE) quality control of 36mer TaqMan oligonucleotide.

## HPLC Purification Strategy

Retention of dye-labeled oligonucleotides is governed by the hydrophobicity of the dye. This is demonstrated on the purification of three different TaqMan probes (Figure 3). Non-labeled oligonucleotides elute first (1), followed by FAM-labeled failure sequences (2). TAMRA-only labeled failure fragments (3) elute before the

dually-labeled target product (4). Other peaks are mostly un-conjugated TAMRA dye. Target product fraction appears to be a group of co-eluting peaks. The partial separation is caused by the differences in HPLC retention of dye isomers. Mass spectrometry analysis confirmed that the collected product is homogeneous (one molecular mass).

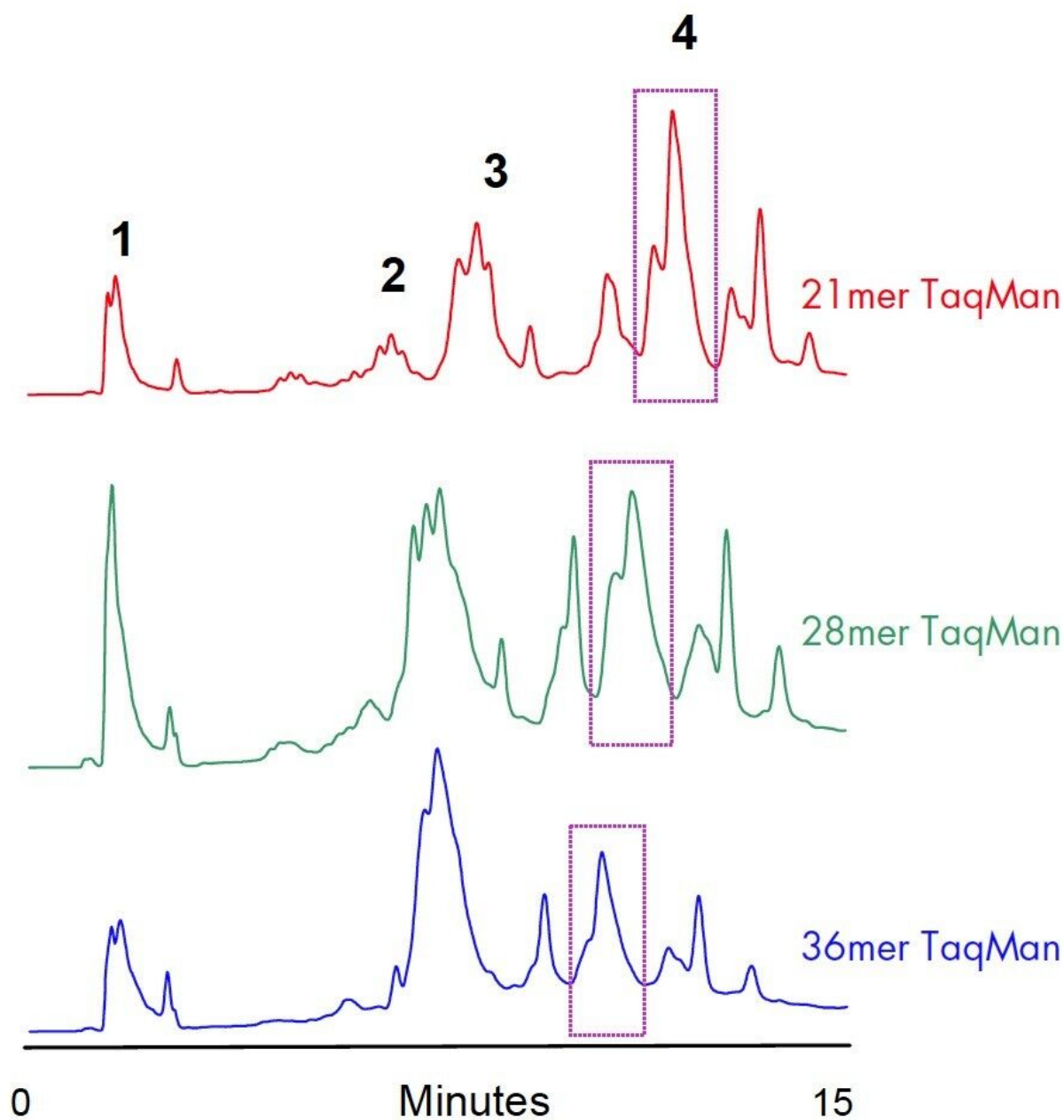


Figure 3. HPLC purification of TaqMan Probes.

In order to select the collection time window, we utilized real-time UV-VIS spectra monitoring along with the 260 nm UV detection (Figure 1). The typical maxima at UV-VIS spectra indicated the elution of non-labeled, singly labeled and dually-labeled products (three  $\lambda_{\text{max}}$  were present in the spectrum). Because of the similar pattern of elution, the purification of different length TaqMan probes becomes a routine for experienced user. Triethylammonium acetate/ACN buffer is volatile. Collected fractions are simply dried down and oligonucleotides are ready for assays.

---

## Featured Products

Alliance HPLC System <<https://www.waters.com/534293>>

WA20772, June 2003