

# OliPure

## 6-FAM / BHQ1 probe purification kit manual

Protocol for removal of contaminants from  
Kilobaser ONE XT synthesized **dual labeled 6-FAM / BHQ1 probes.**

### KIT CONTENTS

- 10 OliPure spin columns
- Solvent #1: 1,5mL 0,1M TEAA in H2O
- Solvent #2: 1,5mL 25% ACN in aqueous 0,1M TEAA
- Solvent #3: 1,5mL distilled H2O
- Solvent #4: 1,5mL 50% ACN in H2O

**Store at room temperature.**

## Purification Kit Introduction & Required Tools

Any crude probe contains traces of salts, dyes and degenerated probes that may interfere with downstream applications. The OliPure spin column kit for dual labeled 6-FAM / BHQ1 probes is optimized to remove the above mentioned contaminants by spin column based hydrophobic-interaction-chromatography.

### LAB EQUIPMENT

- Pipet which covers 40µL
- Centrifuge (suitable for 0.2mL and 1.5mL tubes)
- Vortex mixer
- Two 1.5mL reaction tubes
- One 0.2mL reaction tube

## Step By Step Instruction

### Clean-up of dual-labeled 6-FAM / BHQ1 probe

01. Take your 0,2mL tube with crude probe and resuspend it in 40µL of **solvent #1** provided (0,1M TEAA in H2O).
02. Vortex for 2 minute and briefly spin down in centrifuge.  
**The dissolved probe appears pale to deep purple and / or green**
03. Take a spin column, unscrew its top, remove the red rubber on the bottom and place it in a 1.5mL tube.
04. Pipett the full 40µL of the probe into the spin column.
05. 1st Spin: Place the 1.5 ml tube with the lid open and the spin column inserted into your centrifuge. Centrifuge at 500 - 10,000 × g for minimum 10 sec.  
**The eluent appears clear. Spin column appears pale purple and / or green; the probe has bond to the column.**
06. Discard the eluent and place tube plus spin column back into the centrifuge.
07. 2nd Spin: Add 120µL of **solvent #2** provided (25% ACN in aq. 0,1M TEAA) to the column and centrifuge at 500 - 10,000 × g for minimum 10sec.  
**The eluent appears pale to strong green. Free 6-FAM and degenerated probes have eluted.**
08. Discard the eluent and place tube plus spin column the back into the centrifuge.
09. 3rd Spin: Add 120µL of **solvent #3** provided (distilled H2O) to the column and centrifuge at 500 - 10,000 × g for minimum 10 sec.  
**The eluent appears clear.**
10. Discard the eluent and the 1.5mL tube. Place the spin column in a **fresh 1.5mL tube** to collect the purified probe and place it into the centrifuge.
11. 4th spin: Add 40µL of **solvent #4** provided (50% ACN in H2O) to the column and centrifuge at 500 - 10,000 × g for minimum 10sec. Discard the spin column after checking its colour.  
**The eluted probe appears purple. The spin column appears white.**
12. Remove the solvent by drying the probe, either by heating it to 50°C until dry or by transferring the probe to a 0,2mL reaction tube and using the drying protocol of the Kilobaser Synthesizer.

### QUANTIFICATION

Via microvolume spectrophotometer: For 6-FAM probes with BHQ-1, use following formula:  
Concentration in pmol/µL = Abs 534nm/0,034

### VIDEO

Use the link below or QR code to view the video tutorial.  
[oligo.link/purevid](https://oligo.link/purevid)



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