



## **RetroPrep Dye Removal Kit**

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## **RetroPrep Dye Removal Kit**

### Description

RetroClean Dye Removal kit is designed to efficiently remove unincorporated dye terminators from sequencing reactions. The removal of dye terminators is important to prevent the unincorporated dye from interfering with the sequencing results. RetroClean procedure uses the gel filtration technology that is generally acknowledged as the best method of removing salts, dye terminators, and any other small molecule contaminants. When the sequencing reaction mixtures are applied to the RetroClean columns, the dye terminators diffuse into the pores and are retained in the gel-filtration material, while the sequencing DNA fragments are excluded and collected in the flow-through. This method is faster, more reproducible, and generates better quality sequence data than most ethanol precipitation method.

### Key benefits

- Dye-terminator removal in 10 minutes
- Fast procedure with few centrifugation steps
- Efficiently remove all unincorporated dye from sequencing reactions
- No prehydration or ethanol precipitation necessary
- Generate high quality automated sequencing data

### Contents

- 100 RetroClean Columns
- 100 Collection Tubes

### Storage and Stability

The RetroClean columns should be stored at +4°C. These columns should be stable at this temperature for a period of 6 months.



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## **Protocol**

### **Important consideration before starting:**

The RetroClean Column must be centrifuged at 1500 x g. The appropriate speed can be calculated as follows:  $\text{rpm} = 1000 \times \sqrt{1500/r}$ , (r = radius of rotor in mm)

### **Speed corresponding or 1500 x g of commonly used microcentrifuges:**

<b>Microcentrifuge</b>	<b>Speed</b>
Eppendorf Centrifuge 5415C	4300 rpm
Eppendorf Centrifuge 5417C	3800 rpm
Hereaus Biofuge 15	4000 rpm
Httich Mikro 24-48	3670 rpm

- 1. Place the RetroClean column in one of the collection tubes provided and centrifuge for 1500 x g for 2 min.**
- 2. Carefully transfer the RetroClean column to the clean microcentrifuge tube and discard the collection tube. Slowly apply the sequencing reaction (10-20  $\mu\text{l}$ ) to the gel bed.**

Note: Pipet the sequencing reaction directly to center of the gel bed surface. The sample should be pipetted slowly so that the drops are absorbed into the gel. Do not pipet the sample to side of the column.

- 3. Centrifuge the column for 2 minutes.**

The eluate contains the purified sequencing reaction.

- 4. Remove the spin column from the microcentrifuge tube and dry the purified sample in the vacuum centrifuge according to the instructions provided with the DNA sequencer.**