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	PIS-051 AGEK-Preps	Version: 002 Effective Date: 07/06/20 Author: Lacey LaFave CO#: 062520-1

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Product Information Sheet and Protocol

Concentration: Not Applicable

Storage and Handling: Store at room temperature upon arrival.

Ordering Information:

Item Number	Preparations	Number of Tubes and Volumes
AGEK-50	50	AGEK Extraction Buffer: 1x75mL, AGEK Activation Buffer: 1x6mL, AGEK Washing Buffer: 1x16mL (Add 64mL Ethanol gives 80mL final Volume), AGEK Elution Buffer: 1x5mL, AGEK Spin Columns & Collection Tubes: 1x50
AGEK-250	250	AGEK Extraction Buffer: 2x185mL, AGEK Activation Buffer: 1x30mL, AGEK Washing Buffer: 2x40mL (Add 160mL Ethanol gives 200mL final Volume to each bottle), AGEK Elution Buffer: 1x25mL, AGEK Spin Columns & Collection Tubes: 1x250 each

Product Description:

Empirical Bioscience Agarose Gel Extraction Kit is designed to extract high-yield DNA from agarose gels with simultaneous removal of primer dimers, nucleotides, proteins, salt, agarose, ethidium bromide, and other impurities. The preparation is based on a silica-membrane technology for binding DNA in high-salt and elution in low-salt buffer. The kit provides a simple and efficient way to purify DNA in the size range from 100 bp to 10 kb. It requires no organic extractions or precipitation and guarantees high and stable recovery rates.

Kit contents:

- Extraction Buffer
- Activation Buffer
- Washing Buffer (add 96-99% Ethanol as indicated)
- Elution Buffer
- Spin Columns
- 2 ml Collection Tubes.

Additional Materials not provided:

- 96-99% Ethanol
- Isopropanol (optional)
- 1.5 ml microtubes

Preparation Procedure:

The DNA purification follows a simple binding, washing and eluting procedure. Before starting, add 96-99% Ethanol to the Washing Buffer as indicated on the bottle. The additional use of Isopropanol is recommended for fragments smaller than 200 bp or larger than 5 kb.

- For AGEK-50 Kits add 64mL of 96-99% Ethanol to the Washing Buffer Bottle and indicate addition on the bottle.
- For AGEK-250 Kits add 160mL of 96-99% Ethanol to each Washing Buffer Bottle and indicate addition on the bottle

*This product is intended for Research Use Only. This product is manufactured under ISO13485:2016 Quality System Requirements and is available for use as a Raw Material for use in IVD applications. Please contact Empirical Bioscience for further details.

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Protocol

Excision of the Gel:

- Cut the area of gel containing the DNA fragment.
- Transfer the excised gel to a clean 1.5 ml microtube.

Sample Preparation:

- Add 3 volumes Extraction Buffer to 100 mg of the sliced gel. (3 μ l for every 1mg).
 - For gels containing >2.5% agarose, add 6 volumes Extraction Buffer.
 - For DNA fragment sizes smaller than 200 bp or larger than 5 kb and to enhance yield, add 1 volume Isopropanol per 100 mg gel to dissolved gel and mix well.
- Incubate at 60°C for 10 min with occasional mixing to ensure gel dissolution.

Column Activation:

- Place a Spin Column into a 2 ml Collection Tube.
- Add 100 μ l of Activation Buffer into the Spin Column.
- Centrifuge at 10,000 g for 30 sec in a micro-centrifuge.

Column Loading:

- Apply the sample mixture from sample preparation into the activated Spin Column.
- Centrifuge at 10,000 g for 30 sec in a micro-centrifuge.
- Discard the flow-through.

Column Washing:

- Place the DNA loaded Spin Column into the used 2 ml tube.
- Apply 700 μ l of Washing Buffer to the Spin Column.
- Centrifuge at 10,000 g for 30 sec and discard the flow-through.

Optional Secondary Washing: Recommended only for DNA >200 bp, if highly purified DNA (for DNA sequencing, transfection, etc.) is required.

- Add 700 μ l of Washing Buffer to the Spin Column.
- Centrifuge at 10,000 g for 30 sec and discard the flow-through.
- Centrifuge again for 2 min to remove residual Washing Buffer.

Elution:

- Place the Spin Column into a clean 1.5 ml microtube (not provided).
- Add 30-50 μ l Elution Buffer or dd-water to the center of the column membrane.
- Incubate at room temperature for 1 min.
- Centrifuge at 10,000 g for 1 min to elute DNA.

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