

Instructions For Use

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iX-Pure™

Dye Terminator Cleanup Kit

For BrilliantDye™ and BigDye® v1.1 and v3.1 Chemistries



NimaGen.

Innovators in
DNA Sequencing
Technologies

Product and Company Information

iX-Pure™ DyeTerminator Cleanup Kit



IXP-100, IXP-1000, IXP-2500, IXP-40K



Research Use Only



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QUALITY MANAGEMENT SYSTEM

ISO 9001:2015 FM 711484
ISO 13485:2016 MD 711483

Symbols Used on Product Labels

| Symbol | Description |
|---|---|
|  | Manufacturer |
|  | Use-by date |
|  | Lot number |
|  | Reference number |
|  | Research Use Only |
|  | Temperature limit for storage |
|  | Contains sufficient for <n> tests |
|  | Matrix code containing the reference number, lot number and use-by date |

Product Description

The iX-Pure™ Dye Terminator Removal Kit effectively purifies BrilliantDye™ or BigDye® dye terminator cycle sequencing reactions, by removing unwanted components such as unincorporated dye terminators, dNTPs and salt ions. This prevents their co-injection with your sequencing products.

The iX-Pure™ resin-based workflow does not require repeated wash steps, minimizing sample loss from either long or short fragments of extension products. Typically, resin is added to the finished Sanger cycle sequencing products and vortexed, allowing the resin to capture and immobilize unincorporated dye terminators and salt-ions. The captured components are then moved to the bottom of the reaction vessel by brief centrifugation. The purified dye-labeled extension products remaining in the supernatant are then injected directly from the supernatant into the Genetic Analyzer for capillary electrophoresis (CE).

The kit consists of two reagents:

- iX-Pure™ Resin – Binds unincorporated dye terminators and free salts from the post-sequencing reaction.
- iX-Pure™ Activator - Enhances the performance of the iX-Pure™ Resin solution and stabilizes the post-purification reactions.

iX-Pure™ Dye Terminator Cleanup kits are widely adopted as proven, high-quality reagents for laboratories using 310, 3100, 3130, 3500, 3730 and SeqStudio™ Genetic Analyzers and are fully compatible with BigDye XTerminator™ run modules.

Kit Contents and Storage

iX-Pure™ Dye Terminator Cleanup Kits include ready-for-use reagents for purification of 100 up to 160,000 cycle sequencing reactions, using a 96-well (10 or 20 µL reactions) or 384-well (5 µL reactions) plate format:

| Reference | # Reactions (96-well, 20 µL) | # Reactions (384-well, 5 µL) | Volume iX-Pure™ Resin | Volume iX-Pure™ Activator | Storage |
|-----------|---------------------------------|---------------------------------|-----------------------------|---------------------------------|--|
| IXP-100 | 100 | 400 | 2 mL | 9 mL | Store kit at 4°C, protected from light |
| IXP-1000 | 1,000 | 4,000 | 20 mL | 90 mL | |
| IXP-2500 | 2,500 | 10,000 | 50 mL | 225 mL | |
| IXP-40K | 40,000 | 160,000 | 800 mL | 3,600 mL | |

| Contents | Reference IXP-100 | Reference IXP-1000 | Reference IXP-2500 | Reference IXP-40K |
|--------------------|----------------------|-----------------------|-----------------------|----------------------|
| iX-Pure™ Resin | IXP-100R | IXP-1000R | IXP-2500R | IXP-40000R |
| iX-Pure™ Activator | IXP-100A | IXP-1000A | IXP-2500A | IXP-40000A |

Important Notices

- When loading plates directly into the Genetic Analyzer use the BigDye Xterminator™ Purification Kit run modules specified for your instrument. Modules are available at www.thermofisher.com/sangerpatches. These BigDye Xterminator™ run modules adjust the sample injection height to prevent the capillary array from going into the iX-Pure™ material at the bottom of the wells, potentially affecting the data.
- Before pipetting, make sure the reagents are mixed until homogeneous.
- Do not use formamide or heat denaturing on samples containing iX-Pure™ reagents.
- For 384-well reactions with volumes less than 5 µL, add diH₂O to bring volumes to 5 µL before adding iX-Pure™ reagents.
- For 96-well reactions with volumes less than 10 µL, add diH₂O to bring volumes to 10 µL before adding iX-Pure™ reagents.
- When particles are visible in the iX-Pure™ Activator solution, heat the solution to 37°C and gently mix to re-dissolve. Cool to room temperature before using.

Required Materials, Not Included

| Description |
|---|
| diH ₂ O |
| 96- or 384-well plates, compatible with Genetic Analyzer |
| (Multichannel) Pipettes, including disposable filter tips; wide-bore tips are recommended |
| Pipettes, including disposable conventional tips; wide-bore tips are recommended |
| Empty and clean container for mixing iX-Pure™ Resin and Activator |
| Plate spinner or centrifuge |
| Vortex |

General Precautions

Read the Material Safety Data Sheet (MSDS) and follow the handling instructions. Adhere to good laboratory practice and wear protective eyewear, gloves and lab coat when handling the reagents (resin or activator) supplied in this kit. Wash body parts with ample amount of water immediately if they come in contact with the reagents. Seek medical help if needed.

Protocol

The iX-Pure™ protocol provides two workflows for cycle sequencing reaction purification, preparing samples for capillary electrophoresis on Genetic Analyzers:

- Premix pipetting (premixing iX-Pure™ Resin and Activator)
- Sequential pipetting (subsequent addition of iX-Pure™ Activator and Resin)

Premix Pipetting

1. Based on your plate and reaction size, calculate the volume of iX-Pure™ Resin and iX-Pure™ Activator required. The volumes below include an additional 10% to account for dead volume and pipetting loss.

For 96-well plate, 10 µL reactions

| Reagent | Volume/ well | Volume/ plate | # Reactions | Total Volume |
|--------------------|-----------------|------------------|--------------|-----------------|
| iX-Pure™ Resin | 11 µL | 1056 µL | <your notes> | <your notes> |
| iX-Pure™ Activator | 49.5 µL | 4752 µL | <your notes> | <your notes> |

For 96-well plate, 20 µL reactions

| Reagent | Volume/ well | Volume/ plate | # Reactions | Total Volume |
|--------------------|-----------------|------------------|--------------|-----------------|
| iX-Pure™ Resin | 22 µL | 2112 µL | <your notes> | <your notes> |
| iX-Pure™ Activator | 99 µL | 9504 µL | <your notes> | <your notes> |

For 384-well plate, 5 µL reactions

| Reagent | Volume/ well | Volume/ plate | # Reactions | Total Volume |
|--------------------|-----------------|------------------|--------------|-----------------|
| iX-Pure™ Resin | 5.5 µL | 2112 µL | <your notes> | <your notes> |
| iX-Pure™ Activator | 24.75 µL | 9504 µL | <your notes> | <your notes> |

2. Vortex the iX-Pure Resin container at maximum speed for at least 10 seconds, or until it is homogeneous.
3. Using a wide-bore pipette tip, add the calculated volume of iX-Pure™ Resin to a clean container.
4. Using a conventional pipette tip, add the calculated volume of iX-Pure™ Activator to the clean container.
5. Mix the reagents until homogeneous.

NOTE: This premix can be stored at 4°C for up to 5 days. Make sure to mix well prior to use.

- Follow the cycle sequencing protocol. When the reaction is complete, centrifuge the sequencing reaction plate for 1 minute to spin down the contents.
- To each well, add the volume of premix specified below.

| Plate Type | Reaction Volume/Well | Pre-Mix Volume/Well |
|------------|----------------------|---------------------|
| 96-well | 10 μ L | 55 μ L |
| 96-well | 20 μ L | 110 μ L |
| 384-well | 5 μ L | 27.5 μ L |

- Follow instructions “After Pipetting”.

Sequential Pipetting

- Follow the cycle sequencing protocol. When the reaction is complete, centrifuge the sequencing reaction plate for 1 minute to spin down the contents.
- To each well, add the volume of iX-Pure™ Activator specified below.

| Plate Type | Reaction Volume/Well | Pre-Mix Volume/Well |
|------------|----------------------|---------------------|
| 96-well | 10 μ L | 45 μ L |
| 96-well | 20 μ L | 90 μ L |
| 384-well | 5 μ L | 22.5 μ L |

- Vortex the iX-Pure™ Resin container at maximum speed for at least 10 seconds, or until the Resin solution is homogeneous.
- To each well, add the volume of iX-Pure™ Resin specified below, using a wide-bore pipette tip.

| Plate Type | Reaction Volume/Well | Pre-Mix Volume/Well |
|------------|----------------------|---------------------|
| 96-well | 10 μ L | 10 μ L |
| 96-well | 20 μ L | 20 μ L |
| 384-well | 5 μ L | 5 μ L |

- Follow instructions “After Pipetting”.

After Pipetting (Capillary Electrophoresis)

1. Seal the reaction plates using heat seals or adhesive films. Verify that each well is sealed.
2. Vortex the reaction plate for 30 minutes using the following conditions:

| Vortexer | Speed |
|----------------------------|-----------|
| Eppendorf MixMate 2600 rpm | 2600 rpm |
| IKA MS3 Digital | 2000 rpm |
| IKA Vortex 3 | Setting 5 |
| Genie 2 Digital Vortex | 2000 rpm |
| Taitec MicroMixer E-36 | Maximum |

3. Spin the plate at 1000 x g for at least 1 minute in a swing-bucket centrifuge.
4. When using the BigDye XTerminator™ run module, remove the seal from the reaction plate and directly load it into the Genetic Analyzer for capillary electrophoresis.

When using normal run modules, transfer 20 µL of supernatant to a clean plate and load into the Genetic Analyzer.

Customer Support

For technical assistance, please contact us at techsupport@nimagen.com.

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