Instructions For Use

Version: 2.0 *Ref: IFU-IXPURE* *Revision date: 2023-06-23*

iX-Pure™

DyeTerminator 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

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

Research Use Only

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Symbols Used on Product Labels and in Instructions For Use

Symbol	Description
	Manufacturer
	Use-by date
	Lot number
	Reference number
	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™ DyeTerminator Removal Kit effectively purifies BrilliantDye™ or BigDye® 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.

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™ DyeTerminator Cleanup Kits are widely adopted as proven, high-quality reagents for laboratories using 3130, 3500, 3730 and SeqStudio™ Genetic Analyzers and are fully compatible with BigDye XTerminator™ run modules.

Kit Contents and Storage

iX-Pure™ DyeTerminator Cleanup Kits include ready-for-use reagents for purification of 100 up to 160000 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 (96-well, 10 µL)	# Reactions (384-well, 5 µL)	Volume iX-Pure™ Resin	Volume iX-Pure™ Activator	Storage
IXP-100	100	200	400	2 mL	9 mL	Store kit at 4 °C, protected from light
IXP-1000	1000	2000	4000	20 mL	90 mL	
IXP-2500	2500	5000	10000	50 mL	225 mL	
IXP-40K	40000	80000	160000	800 mL	3600 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 96-well reactions with volumes less than 10 µL, add diH_2O to bring volumes to 10 µL before adding iX-Pure™ reagents.
- For 384-well reactions with volumes less than 5 µL, add diH_2O to bring volumes to 5 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_2O
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 the Resin solution 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.

6. Follow the cycle sequencing protocol. When the reaction is complete, centrifuge the sequencing reaction plate for 1 minute to spin down the contents.

7. To each well, add the volume of premix specified below.

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

8. Follow the instructions “After Pipetting”.

Sequential Pipetting

1. Follow the cycle sequencing protocol. When the reaction is complete, centrifuge the sequencing reaction plate for 1 minute to spin down the contents.

2. To each well, add the volume of iX-Pure™ Activator specified below.

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

3. Vortex the iX-Pure™ Resin container at maximum speed for at least 10 seconds, or until the Resin solution is homogeneous.

4. 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 µL	10 µL
96-well	20 µL	20 µL
384-well	5 µL	5 µL

5. Follow the 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:

Vortex	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.

Revision History

Section	Summary of changes	Version	Date
All	Not applicable.	1.3	2019-03-05
All	New layout. New introduction (Product Description). Kit Contents and Storage. General Precautions.	2.0	2023-06-23

Legal Notice

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