iX-Pure™ DyeTerminator Cleanup Kit

Quick Reference Guide

Version: 1.3
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Product and Company Information

Product name: iX-Pure™ DyeTerminator Cleanup kit

Product use: For Research Use Only

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Description

The iX-Pure™ DyeTerminator Cleanup kit purifies the cycle-sequencing reaction by removing unwanted components such as salt ions, unincorporated dye terminators and dNTPs. This prevents their co-injection with your sequencing products.

The kit consists of two reagents:

- iX-Pure Resin
- iX-Pure Activator

These reagents can be added as a premix or sequentially.

Cleanup is complete in under 40 minutes and requires less than 10 minutes of hands-on time.

Kit Content

p/n	Reactions (20µl)	Volume of kit reagent (mL)	
		iX-Pure Resin	iX-Pure Activator
IXP-100	100	2	9
IXP-1000	1,000	20	90
IXP-2500	2,500	50	225
IXP-40K	40,000	800	3600

Important notes

When loading plates directly into the CE instrument use the BigDye XTerminator Purification Kit run modules specified for your instrument. The run modules are available at www.thermofisher.com/sangerpatches.

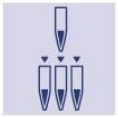
These BDX run modules adjust the sample injection height to prevent the capillary array from going into the iX-Pure material at the bottom of the wells, potentially affecting the data.

- 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 water to bring volumes to 5 µl before adding iX-Pure reagents.
- For 96-well reactions with volumes less than 10 µl, add water to bring volumes to 10 µl before adding iX-Pure reagents.
- If particulates are visible in the Activator solution, heat the solution to 37˚C and mix te re-dissolve. Cool to room temperature before using.

Workflow

iX-Pure workflow

1 Add iX-Pure



Add iX-Pure™ reagent
to your finished reaction

2 Incubate

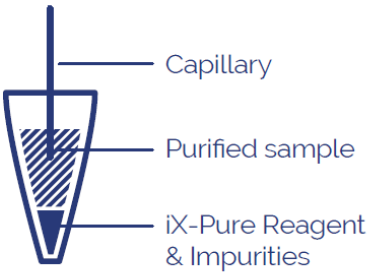
Vortex

Centrifuge

3 Injection

Ready for
sequencing

iX-Pure method



Protocol (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, 10µl reactions:

Reagent	Volume / Well (µl)	Volume / Plate (µl)	Number of reactions	Final volume
iX-Pure Activator	49,5 µl	4752 µl		
iX-Pure Resin	11 µl	1056 µl		

For 96-well plate, 20µl reactions:

Reagent	Volume / Well (µl)	Volume / Plate (µl)	Number of reactions	Final volume
iX-Pure Activator	99 µl	9504 µl		
iX-Pure Resin	22 µl	2112 µl		

For 384-well plate, 5µl reactions

Reagent	Volume / Well (µl)	Volume / Plate (µl)	Number of reactions	Final volume
iX-Pure Activator	24,75 µl	9504 µl		
iX-Pure Resin	5,5 µl	2112 µl		

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.
   (This premix can be stored at 4˚C for up to 5 days. Make sure to mix well before 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. Add the premix to each well, volumes are stated below.

Reaction volume per well	Volume of Premix / Well
96-well, 10 µl	55,0 µl
96-well, 20 µl	110,0 µl
384-well, 5 µl	27,5 µl

8. Follow instructions "Protocol (After pipetting)" on page 5.

Protocol (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.

Reaction volume per well	Volume of iX-Pure Activator / Well
96-well, 10 µl	45,0 µl
96-well, 20 µl	90,0 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 it is homogeneous.
4. To each well, add the volume of iX-Pure **Resin** specified below. Using a wide-bore pipette tip.

Reaction volume per well	Volume of iX-Pure Resin / Well
96-well, 10 µl	10,0 µl
96-well, 20 µl	20,0 µl
384-well, 5 µl	5,0 µl

5. Follow instructions "After pipetting" on page 5.

Protocol (After Pipetting)

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 rmp
IKA MS3 Digital	2000 rpm
IKA Vortex 3	Setting 5
Digital Vortex Genie 2	2000 rmp
Taitec MicroMixer E-36	Maximum

3. Spin the plate at 1000 x *g* for at least one minute in a swing-bucket centrifuge.
4. When using the BigDye XTerminator run module, remove the seal from the reaction plate and place in CE instrument. When using normal run modules, transfer 20µl of supernatant to a clean plate and place in instrument.