

XT Purification Kit User Manual

Sequencing workflow

- ♦ STEP 1: Add XT Purification Reagents
- ♦ STEP 2: Seal reaction plate
- ♦ STEP 3: Vortex for 30 minutes
- ♦ STEP 4: Centrifuge

Run purified samples on your DNA Analyzer

Overview

The XT Purification Kit sequesters cycle-sequencing reaction components such as salt lons,unincorporated dye terminators, and dNTPs to prevent their co-injection with dye-labeled extension products into a CE DNA analyzer. The XT Purification reagents can be pipette separately and sequentially into reaction plate, or premixed together before being pipette into a reaction plate.

Ordering Information

Refer to the XT Purification Kit Protocol for recommended vortexers and required accessories.

| | | Volume of Each | | | |
|--|-----------|----------------|----------|----------------|--|
| | Kit Size | Resin | Solution | Catalog Number | |
| | 50preps | -1ml | 4.5ml | 8500050 | |
| | 1000preps | 20ml | 90ml | 8502010 | |
| | 5000preps | 100ml | 450ml | 8502050 | |
| | | GENE | FCHNC | DOGY | |

Important Tips

- ♦ When you pipette directly from the Solution bottle:
 - Before pipetting, mix the Solution until homogeneous,
 - Use wide-bore pipette tips,

Avoid pipetting near the surface of the liquid,

When you seal the reaction plate, verify that each well is sealed.

- ✤ To achieve optimum performance, use a recommended vortexer and follow the protocol when you vortex the reaction plate.
- \diamond When you load plates into the CE instrument:

Do not heat-denature or use Formamide with samples containing XT Purification reagents. Use the ABI run modules specified for your instrument and plate type.

| STEP | ACTION | | | | | |
|------|--|--|--|--|--|--|
| 1 | Centrifuge the Follow the cycle-sequencing protocol. When the reaction is complete, centrifuge sequencing the reaction plate for 1 minute to spin down plate contents. | | | | | |
| | ReactionIMPORTANT! You may need to decrease the amount of DNA template in the | | | | | |
| | plates. | sequencing reactions to compensate for increased signal strength. See "DNA Quantity Guidelines" on page 5. | | | | |

Procedure for Sequential Pipetting



| 2 | Add the | To each well of the reaction plate, add the volume of the Solution specified below, | | | | | |
|---|-----------------|---|--|----|--|--|--|
| | Solution to the | using a conventional pipette tip. | | | | | |
| | reaction plates | Make sure there are no particulates in the Solution before pipetting. If particulates | | | | | |
| | | are present, heat the Solution to 37 $^\circ\!\!\mathbb{C}$ and mix to redissolve. Cool to roon | | | | | |
| | | temperature before using. | | | | | |
| | | Plate Type and Reaction Volume of the Solution/Well (µI) | | | | | |
| | | Volume/Well | | | | | |
| | | 384-well,5 µl | 22.5 | | | | |
| | | 96-well,10 µl | 45.0 | | | | |
| | | 96-well,20 µl | 90.0 | | | | |
| | | IMPORTANT! For 384-well react | ons with reaction volumes less than 5 μl, a | dd | | | |
| | | water to bring the volumes to 5 μ l | pefore adding the Solution. For 96-well reaction | ns | | | |
| | | with reaction volumes less than | 10 μ l, add water to bring the volume to 10 | μl | | | |
| | | before adding the solution. | | | | | |
| 3 | Add the Resin | Add the Resin: | | - | | | |
| | to the reaction | a. Vortex the resin at maximur | n speed for at least 10 seconds, until it | is | | | |
| | plates using a | homogeneous | | | | | |
| | wide-bore | b. Using a wide-bore pipette tip | , add to the reaction plate the volume of t | he | | | |
| | pipette tips | Solution specified below. | | | | | |
| | | Plate Type and Reaction | Volume of the Solution/Well (µI) | | | | |
| | | Volume/Well | | | | | |
| | 1 | 384-well,5 µl | 5.0 | | | | |
| | | 96-well,10 μl 10.0 96-well,20 μl 20.0 | | | | | |
| | | | | | | | |
| 4 | Seal, vortex, | Follow the instructions in "After Pi | betting Is Complete" on page 4. | | | | |
| | load and run | - | CHNOLOGY | | | | |
| | the plates | | | | | | |
| | | | | | | | |

Procedure for Premix Pipetting

Note: The premix is stable only for 5 days. Make only the volume of premix that you will use in 5 days.

| STEP | ACTION | | | | | | | |
|------|------------------------------------|---|--------------------|--------------|-----------|--------------|--|--|
| 1 | Calculate the | Based on your plate and reaction size, calculate the volume of the Solution and | | | | | | |
| | required | Resin needed. | | | | | | |
| | volume of the | Note: All volumes below include an additional 10% to account for dead volume in | | | | | | |
| | Purification | the reagent troug | gh. | | | | | |
| | reagents. | For 384-well pla | te, 5-µl reactions | : | | | | |
| | Volume/Well Volume/Plate Number of | | | | | | | |
| | | Reagent | (µI) | (µI) | Reactions | Needed | | |
| | | Solution 24.75 9504 | | | | | | |
| | | Resin 5.5 2112 | | | | | | |
| | | For 96-well plate, 10-µl reactions: | | | | | | |
| | | Peagent | Volume/Well | Volume/Plate | Number of | Final Volume | | |
| | | Reagent | (µI) | (µI) | Reactions | Needed | | |
| | Solution 49.5 4752 | | | | | | | |
| | Resin 11 1056 | | | | | | | |
| | | For 96-well plate | e, 20-µl reactions | : | | | | |



| | | | 1 | | | 1 | | |
|---|---|--|--|--|---|--|--|--|
| | | Reagent | Volume/Well | Volume/Plate | Number of | Final Volume | | |
| | | liteugent | (µI) | (µl) | Reactions | Needed | | |
| | | Solution | 99 | 9504 | | | | |
| | | Resin | 22 | 2112 | | | | |
| 2 | Combine the | Combine the So | lution and Resin | | | | | |
| | Reagents to | a. Vortex the | e Resin bottle at r | naximum speed f | or the least 10 s | econds, until it is | | |
| | create the | a. Vortex the Resin bottle at maximum speed for the least 10 seconds, until it i homogeneous. | | | | | | |
| | premix | - | | tip or a graduat | ed cylinder, add | the appropriate | | |
| | | volume of | Resin to a clean | container. | - | | | |
| | | IMPORTAN | IT! Avoid pipettin | g near the surfac | e of the liquid. | | | |
| | | c. Using a co | onventional pipet | - te tip or a gradua | ited cylinder, ad | d the appropriate | | |
| | | volume of | the Solution to the | ne container with | the Resin. | | | |
| | | Make sure | e there are no | particulates in t | he Solution be | fore pipetting. If | | |
| | | particulate | s are present, h | eat the Solution | to 37℃ and m | nix to redissolve. | | |
| | | Cool to roo | om temperature l | pefore using. | | | | |
| | | d. Mix the re | eagents until hon | nogeneous. | | | | |
| | | Note: The prem | nix can be stored | l in a clean, cap | ped container a | at 4 ℃ for up to 5 | | |
| | | days. | | | | | | |
| 3 | Centrifuge the | Following the | cycle-sequencin | g protocol. Wh | en the reaction | on is complete | | |
| | | centrifuge the reaction plate for 1 minute to spin down plate contents. | | | | | | |
| | sequencing | centrifuge the re | action plate for 1 | minute to spin d | own plate conte | ents. | | |
| | sequencing reaction plates. | | · · | minute to spin d decrease the a | · / · | | | |
| | | IMPORTANT! Y sequencing rea | ou may need to compe | · · · · | amount of DNA | template in the | | |
| | | IMPORTANT! Y | ou may need to compe | decrease the a | amount of DNA | template in the | | |
| 4 | reaction plates. Add the premix | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent | You may need to ctions to compe nes" on page 5. tional pipette tip, | o decrease the a nsate for increa add to each well | amount of DNA sed signal stre of the reaction | template in the ngth. See "DNA | | |
| 4 | reaction plates.Add the premixto the reaction | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s | add to each well pecified below. | amount of DNA sed signal stre of the reaction | template in the ngth. See "DNA plate the volume | | |
| 4 | reaction plates. Add the premix | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well read | add to each well pecified below. | of the reaction | template in the ngth. See "DNA plate the volume s than 5 μl, ado | | |
| 4 | reaction plates.Add the premixto the reaction | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well reache volumes to 5 p | add to each well pecified below. with reactions with reactions to be the section of the section | amount of DNA sed signal stre of the reaction on volumes les he premix. For | template in the ngth. See "DNA plate the volume s than 5 μl, add 96-well reactions | | |
| 4 | reaction plates.Add the premixto the reaction | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well read ne volumes to 5 p lume less than 10 | add to each well pecified below. | amount of DNA sed signal stre of the reaction on volumes les he premix. For | template in the ngth. See "DNA plate the volume s than 5 μl, ado 96-well reactions | | |
| 4 | reaction plates.Add the premixto the reaction | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol adding the prem | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well reach he volumes to 5 p lume less than 10 hix. | add to each well pecified below. with reactions with reactions to be the section of the section | amount of DNA sed signal stre of the reaction on volumes les he premix. For | template in the ngth. See "DNA plate the volume s than 5 μl, ado 96-well reactions | | |
| 4 | reaction plates.Add the premixto the reaction | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol adding the prem Plate Type | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well read ne volumes to 5 p lume less than 10 nix. and Reaction | o decrease the a insate for increa add to each well pecified below. itions with reacti l before adding t o µl, add water to | amount of DNA sed signal stre of the reaction on volumes les the premix. For bring the volum | template in the ngth. See "DNA plate the volume s than 5 μl, ado 96-well reactions ne to 10 μl before | | |
| 4 | reaction plates.Add the premixto the reaction | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol adding the prem Plate Type Volum | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well reach the volumes to 5 µ lume less than 10 nix. and Reaction me/Well | o decrease the a insate for increa add to each well pecified below. itions with reacti l before adding t o µl, add water to | amount of DNA sed signal stre of the reaction on volumes les he premix. For bring the volum | template in the ngth. See "DNA plate the volume s than 5 μl, ado 96-well reactions ne to 10 μl before | | |
| 4 | reaction plates.Add the premixto the reaction | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol adding the prem Plate Type Volu 384-v | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well read the volumes to 5 µ lume less than 10 hix. and Reaction me/Well well,5 µl | o decrease the a insate for increa add to each well pecified below. itions with reacti l before adding t o µl, add water to | amount of DNA sed signal stre of the reaction on volumes les the premix. For bring the volum | template in the ngth. See "DNA plate the volume s than 5 μl, ado 96-well reactions ne to 10 μl before | | |
| 4 | reaction plates.Add the premixto the reaction | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol adding the prem Plate Type Volue 384-v 96-w | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well reach the volumes to 5 µ lume less than 10 nix. and Reaction me/Well well,5 µl ell,10 µl | add to each well pecified below. tions with reaction before adding to pul, add water to | amount of DNA sed signal stre of the reaction on volumes les the premix. For bring the volum e Solution/Well (27.5 55.0 | template in the ngth. See "DNA plate the volume s than 5 μl, ado 96-well reactions ne to 10 μl before | | |
| 4 | reaction plates.Add the premixto the reaction | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol adding the prem Plate Type Volue 384-v 96-w | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well reac ne volumes to 5 μ lume less than 10 nix. and Reaction me/Well well,5 μl ell,10 μl ell,20 μl | add to each well pecified below. tions with reaction before adding to pul, add water to | amount of DNA sed signal stre of the reaction on volumes les the premix. For bring the volum e Solution/Well (27.5 55.0 110.0 | template in the ngth. See "DNA plate the volume s than 5 μl, add 96-well reactions ne to 10 μl before | | |
| 4 | reaction plates.Add the premixto the reaction | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol adding the prem Plate Type Volur 384-w 96-w IMPORTANT! N | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well reach he volumes to 5 µ lume less than 10 nix. and Reaction me/Well well,5 µl ell,10 µl ell,20 µl Aix the premix a | add to each well pecified below. tions with reactive before adding to pul, add water to Volume of the s needed to ma | amount of DNA sed signal stre of the reaction on volumes les the premix. For bring the volum e Solution/Well (27.5 55.0 110.0 intain a homog | template in the ngth. See "DNA plate the volume s than 5 μl, add 96-well reactions the to 10 μl before | | |
| 4 | reaction plates.Add the premixto the reaction | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol adding the prem Plate Type Volue 384-v 96-w Dispense the p | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well reach the volumes to 5 µ lume less than 10 nix. and Reaction me/Well well,5 µl ell,10 µl ell,20 µl Aix the premix a remix within 1 r | add to each well pecified below. tions with reaction before adding to pul, add water to | amount of DNA sed signal stre of the reaction <i>OGY</i> on volumes les the premix. For bring the volum e Solution/Well (27.5 55.0 110.0 intain a homog | template in the ngth. See "DNA plate the volume s than 5 μl, add 96-well reactions the to 10 μl before | | |
| | reaction plates. Add the premix to the reaction plates. | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol adding the prem Plate Type Volur 384-v 96-w 96-w Dispense the p reagents in the p | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well reach the volumes to 5 μ lume less than 10 nix. and Reaction me/Well well,5 μl ell,10 μl ell,20 μl Aix the premix a remix within 1 r oipette tip. | add to each well pecified below. tions with reactive before adding to before adding to befo | amount of DNA sed signal stre of the reaction <i>O G Y</i> on volumes les the premix. For bring the volum e Solution/Well (27.5 55.0 110.0 intain a homog ation to avoid s | template in the ngth. See "DNA plate the volume s than 5 μl, add 96-well reactions the to 10 μl before (μl) eneous solution separation of the | | |
| 4 | reaction plates. Add the premix to the reaction plates. plates. Seal, vortex, | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol adding the prem Plate Type Volur 384-v 96-w 96-w Dispense the p reagents in the p | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well reach the volumes to 5 μ lume less than 10 nix. and Reaction me/Well well,5 μl ell,10 μl ell,20 μl Aix the premix a remix within 1 r oipette tip. | add to each well pecified below. tions with reactive before adding to pul, add water to Volume of the s needed to ma | amount of DNA sed signal stre of the reaction <i>O G Y</i> on volumes les the premix. For bring the volum e Solution/Well (27.5 55.0 110.0 intain a homog ation to avoid s | template in the ngth. See "DNA plate the volume s than 5 μl, add 96-well reactions the to 10 μl before (μl) eneous solution separation of the | | |
| | reaction plates. Add the premix to the reaction plates. | IMPORTANT! Y sequencing rea Quantity Guideli Using a convent of the thoroughly IMPORTANT! F water to bring th with reaction vol adding the prem Plate Type Volur 384-v 96-w 96-w Dispense the p reagents in the p | You may need to ctions to compe- nes" on page 5. tional pipette tip, y mixed premix s for 384-well reach the volumes to 5 μ lume less than 10 nix. and Reaction me/Well well,5 μl ell,10 μl ell,20 μl Aix the premix a remix within 1 r oipette tip. | add to each well pecified below. tions with reactive before adding to before adding to befo | amount of DNA sed signal stre of the reaction <i>O G Y</i> on volumes les the premix. For bring the volum e Solution/Well (27.5 55.0 110.0 intain a homog ation to avoid s | template in the ngth. See "DNA plate the volume s than 5 µl, add 96-well reactions the to 10 µl before (µl) eneous solution. separation of the | | |

After Pipetting Is Complete

| STEP | ACTION |
|---------|--------|
| • • = • | |



| | GENE TECHNOLOG | | | | | | |
|---|-----------------|--|---|-----------------|------------------------|-------------------------|--------------|
| 1 | Seal the | Seal the plate | - | | | | |
| | reaction | ■ A heat seal at 160 °C for 2 seconds | | | | | |
| | plates. | or | | | | | |
| | | ■ MicroAmp Clear Adhesive Films or any other good adhesive films. Verify that | | | | | |
| | | each well is sealed. | | | | | |
| | | IMPORTANT! If you are using an ABI 3730 DNA Analyzer and plan to use direct | | | | | |
| | | injection, only | injection, only ABI Heat Seal Film for Sequencing and Fragment Analysis Sam Plates is supported. | | | | |
| | | Plates is supp | | | | | |
| | | | | | | | |
| 2 | Vortex the | vortex the rea | action plate for 30 mi | | | | |
| | reaction | | Vortexer | | te Type | Speed | _ |
| | plates. | | Digital vortex-Genie 2 | 2 | 6-well | 1800 rpm | |
| | | | g | 38 | 34-well | 2000 rpm | |
| | | | Eppendorf MixMate | 38 | 34-well | 2600 rpm | |
| | | | IKA MS3 Digital | | Either | 2000 rpm | |
| | | | IKA Vortex 3 | | Either | Setting 5 | |
| | | Т | aitec MicroMixer E-3 | 6 I | Either | Maximum | |
| | | Unior | n Scientific Vertical Sl | naker | Either | Setting 100 | |
| | | Note: It is rec | commended that you | pause vortexi | ng after 1 i | minute to verify th | nat the |
| | | contents are | | | | | |
| 3 | Centrifuge the | In a swinging | -bucket centrifuge, sr | oin the plate a | t 1000 xa f | or 2 minutes | |
| Ũ | reaction plates | in a swinging | buoket bentindge, op | | t 1000 Ag I | | |
| | reaction plates | | | | $P \land$ | | |
| 4 | Prepare the | Place the rea | ction plate in The CE | instrument. (1 | o store an | d run the plate lat | ter, see |
| | plates for the | step 6.) | | | | | |
| | instrument run. | Plate Type | Instrument | Seal | | Instructions | |
| | | | | | Pl | ace directly in the | • |
| | | G G | ENE IEC | Heat seal | LOG | instrument. | |
| | | | | | ■ Re | move the clear | |
| | | | | | adhesiv | ve film, replace w | ith a |
| | | | 2720/2720 | MicroAmp | heat se | seal, and then place in | |
| | | | 3730/3730xl | Clear | the instrument. | | |
| | | | | Adhesive | ve ■ Transfer 10 µl of | | |
| | | 384-well | | Film | superna | atant to a clean p | late, |
| | | | | | cover w | vith a septa mat, j | place |
| | | | | | in instru | ıment | |
| | | | 3100/3100Avant, | | Transfe | r 10 µl of superna | atant |
| | | | 3130/3130xl, | | | an plate, cover w | |
| | | | or 310 Genetic | | | nat, then place in | |
| | | | Analyzer | Either | instrum | • | |
| | | | | | | ace directly in the | , |
| | | | | Heat seal | | instrument | - |
| | | | | MicroAmp | | modumont | |
| | | | 3730/3730xl | Clear | Rom | ove the seal, repl | ace |
| | | | | Adhesive | | septa mat, place i | |
| | | | | Film | willas | instrument. | |
| | | 960-well | | FIIII | | | |
| | | | | | | | |



| | | | 3100/3100Avant or 3130/3130xl 310 Genetic Analyzer | Either | Remove the seal, replace with a septa mat, place in the instrument. Transfer 10 µl of supernatant to a clean plate, cover with a septa mat, then place in the instrument. | | |
|---|---|---|---|--------|---|--|--|
| 5 | Select the appropriate run module | Select the appropriate BigDye Xterminator run module for your instrument and plate type. Note: Use standard run modules if you transferred the supernatant to a clean plate after ceptrifuging | | | | | |
| 6 | Run the reaction plates | after centrifuging. Run the plate. If the reaction plates are not run immediately, you can store them under the following conditions: Room temperature – Plates sealed with heat seal film, adhesive film, or septa for up to 48 hours at room temperature (20 to 25 °C). Refrigerated storage – Plates sealed with heat seal film or adhesive film for up to 10 days at 4 °C (recommended). Frozen storage – Plates sealed with heat seal film or adhesive film for up to 10 days at -20 °C | | | | | |

DNA Quantity Guidelines

DNA sequencing reactions purified with the XT Purification Kit result in high signal strength when analyzed on a DNA sequencer. Therefore, when you prepare sequencing samples for purification with the XT Purification reagents, you may need to decrease the amount of DNA template in the sequencing reactions to keep the fluorescence signals on scale during analysis. Use the following table as a guide to the amount of template DNA for the initial cycle sequencing.

IMPORTANT! If you decrease the template concentration, also decrease the amount of any template controls proportionately. For example, if you run a pGEM control, dilute if 1:2 or 1:4 and add only 1 to 2 µl.

| Template Type | DNA Quantity/Reaction(ng) | | Template Type | DNA Quantity/Reaction(ng) | |
|-----------------|---------------------------|--|-------------------------|---------------------------|--|
| PCR products | | | Other types of template | | |
| 100 to 200 bp | 0.5 to 3 | | Single-stranded | DNA 10 to 50 | |
| 200 to 500 bp | 1 to 10 | | Double-stranded | DNA 50 to 300 | |
| 500 to 1000 bp | 2 to 20 | | Cosmid or BAC DNA | 200 to 1,000 | |
| 1000 to 5000 bp | 5 to 40 | | Bacterial genomic DNA | 1,000 to 3,000 | |
| >2000 bp | 10 to 50 | | | | |