

Automated Differex™ System Protocol for the Beckman Coulter Biomek® 3000

Automated Protocol #EP032

DESCRIPTION OF THE BECKMAN COULTER BIOMEK® 3000 METHOD FOR
THE AUTOMATED DIFFEREX™ SYSTEM AND THE AUTOMATED DNA IQ™ AFTER DIFFEREX™ SYSTEM.

All technical literature is available on the Internet at: www.promega.com

Please visit the web site to verify that you are using the most current version of this Automated Protocol.

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1. Description

Sections 1–7 of this document describe the Automated Differex™ System on the Beckman Coulter Biomek® 3000 Automated Laboratory Workstation. To obtain information about methods for human identification applications, visit: www.promega.com/applications/hmid/automation/. General automation guidelines for adaptation to other liquid-handling platforms are provided in Section 7. To troubleshoot chemistry issues, please refer to the *Differex™ System Technical Bulletin #TBD020*.

Note: All Promega Technical Bulletins are available at: www.promega.com/tbs/

2. Product Requirements and Storage Conditions

Note: The Differex™ System was initially developed for use in a manual format. The reagent volumes in this document have been adjusted for the automated method. However, the Differex™ System (Cat.# DC6801, DC6800) does not provide the listed number of sample isolations in the automated format. We recommend that you purchase the reagents as standalone products. The Automated Differex™ System method works in concert with the DNA IQ™ System for genomic DNA purification from differentially extracted samples. DNA IQ™ Resin is **required** for automated differential extraction of samples.

Product	Size	Cat.#
Differex™ Digestion Buffer*	150ml	A8501
Differex™ Separation Solution*	40ml	A8511
Nuclease-Free Water**	50ml	P1193
	150ml	P1195
DNA IQ™ System*	100 reactions	DC6701
	400 reactions	DC6700

*Not for Medical Diagnostic Use.

**For Laboratory Use.

Storage Conditions: Store Differex™ Digestion Buffer, Differex™ Separation Solution, DNA IQ™ Resin and Nuclease-Free Water at 22–25°C.

Items Available Separately

Product	Size	Cat.#
Slicprep™ 96 Device*	10 pack	V1391
2.2ml, Square-Well Deep Well Plate	50/case	V6781
MagnaBot® Flat Top Magnetic Separation Device	1 each	V6041
Proteinase K*	100mg	V3021
DTT	5g	V3151
	25g	V3155
DNA IQ™ Resin**	50ml	A8251
Lysis Buffer**	150ml	A8261
2X Wash Buffer**	70ml	A8271
Elution Buffer**	50ml	A8281

*For Laboratory Use.

**Not for Medical Diagnostic Use.

Standalone reagents sufficient for 4 × 48 (full plate) automated differential extractions:

Differex™ Digestion Buffer	A8501	1 bottle (150ml)
Nuclease-Free Water	P1195	2 bottles (300ml)
Nuclease-Free Water	P1193	1 bottle (50ml)
Differex™ Separation Solution	A8511	1 bottle (40ml)
DNA IQ™ Resin*	DC6700	1 bottle (3ml)

*DNA IQ™ Resin obtained from the 400-reaction DNA IQ™ System (Cat.# DC6700) is sufficient for use with the Automated Differex™ System.

3. Materials to be Supplied by the User

- Centrifuge capable of 1,500 × *g*, fitted with 96-well plate adapters (for a list of centrifuges compatible with the Slicprep™ 96 Device, visit the following web page:
www.promega.com/applications/hmid/profiles/CentrifugeCompatibility.htm)
- Proteinase K (Cat.# V3021)
- DTT (Cat.# V3151, V3155)
- DNA IQ™ Resin (either from DNA IQ™ System [Cat.# DC6701, DC6700] or as a standalone reagent [Cat.# A8251])



See Sections 5.A and 5.B for a list of instrumentation and labware required for the Automated Differex™ System on the Beckman Coulter Biomek® 3000 Workstation.

4. Before You Begin

4.A. Preparation of Solutions

Prior to beginning the automated Differex™ System procedure, prepare the following solutions:

Proteinase K Solution

Dilute Proteinase K to 20mg/ml with Nuclease-Free Water. Freeze unused portions in single-use aliquots at –20°C for up to 2 months.

Digestion Solution

Prepare Digestion Solution just before use. For each sample, you will need 400µl of Digestion Solution, which consists of 25µl of Proteinase K Solution and 375µl of Digestion Buffer. For example, if there are 40 samples, prepare 40 × 400µl = 16ml of Digestion Solution by mixing 40 × 25µl = 1ml of Proteinase K Solution with 40 × 375µl = 15ml of Digestion Buffer. Include an additional 75µl of Proteinase K and 1,125µl of Digestion Buffer for the necessary dead volume for all samples quantities used.

Resin Solution

Prepare Resin Solution prior to running the Automated Differex™ System method. Each sample requires 100µl of Resin Solution, which consists of 14µl of DNA IQ™ Resin and 86µl of Nuclease-Free Water. In addition, a 1,500µl dead volume (210µl DNA IQ™ Resin and 1,290µl Nuclease-Free Water) is needed in the trough. That is:

Resin volume = (# samples × 14µl of DNA IQ™ Resin) + 210µl dead volume

Nuclease-Free Water volume = (# samples × 86µl) + 1,290µl dead volume

For example, if there are 40 samples:

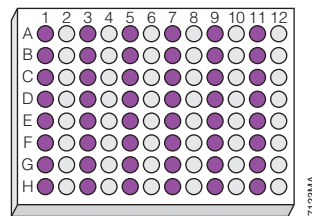
Resin volume = (40 × 14µl of DNA IQ™ Resin) + 210µl dead volume = 770µl

Nuclease-Free Water volume = (40 × 86µl) + 1,290µl dead volume = 4,730µl

Prepare this solution, and mix thoroughly before pouring into the trough.

4.B. Sample Preparation Before Automated Processing

- Sample wells
- Empty wells



Prepare a Slicprep™ 96 Device by removing the U-Shaped Collar and pushing the 96 Spin Basket into the 96 Deep Well Plate. Sexual assault swabs should be placed in columns 1, 3, 5, 7, 9 and 11 of a 96 Spin Basket. To each sample, add 400µl of Digestion Solution, then seal the top of the device with a plate sealer (e.g., Seal & Sample Aluminum Foil Lids Beckman Cat.# 538619). Place the Slicprep™ 96 Device in a water bath or incubator at 56°C for 1.5 hours. After digestion, raise the 96 Spin Basket and insert the U-Shaped Collar between the plate and 96 Spin Basket. Start the automated method while spinning the Slicprep™ 96 Device at 1,500 × g for 10 minutes.

5. Automated Processing Requirements for the Biomek® 3000 Workstation

This section lists the instrument and labware requirements for the Automated Differex™ System method on the Biomek® 3000.

5.A. Beckman Coulter Products Required for the Automated Differex™ System on the Biomek® 3000

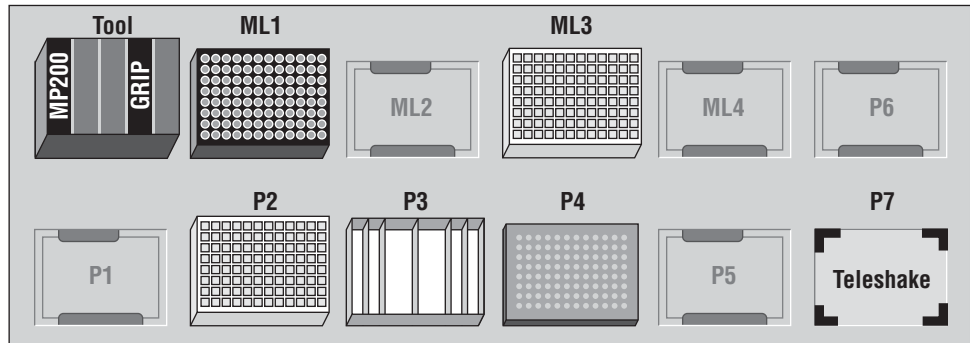
The following is a list of Beckman Coulter parts and their corresponding part numbers that are required for the Automated Differex™ System method on a Biomek® 3000.

Part Description	Quantity	Beckman Coulter Part Number
Biomek® 3000 Workstation, 50/60Hz, 100–240V	1	986120
Biomek® 3000 Automation Controller XP and Monitor with Biomek® System Software	1	A16170
Biomek® 3000 Left Side Module	1	987264
Biomek® 3000 Right Side Module	1	987263
Gripper Tool Kit for Biomek® 3000	1	A09053
MP200 Eight-Tip Tool	1	986146
Tip Rack Holder	1	391910
Gray Labware Holder	4	609120
Frame for Reservoirs	1	372795
Quarter Reservoir	2	372790
Quarter Reservoir, Divided by Length	1	372788
AP96 P250 Barrier Tips	1	717253

5.B. Promega Products Required for Automated Differex™ System on the Biomek® 3000

Part Description	Quantity	Promega Cat.#
MagnaBot® Flat Top Magnetic Separation Device	1	V6041
Slicprep™ 96 Device	1	V1391
2.2ml, Square-Well Deep Well Plate	2	V6781
Shaker Integration Plate	1	V3691
VARIOMAG® Teleshake, (110V, For North American Use Only)	1	V6751

5.C. Biomek® 3000 Initial Deck Configuration



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Figure 1. Biomek® 3000 initial deck configuration.

Position Tool	Tool rack containing MP200 and Gripper tools
Position ML1	Tip rack holder, P250 tips
Position ML2	Empty
Position ML3	Gray labware holder, empty 2.2ml, Square-Well Deep Well Plate ("Archival/Waste Plate")
Position ML4	Empty
Position P6	Empty, V&P Heater Position
Position P1	Empty
Position P2	Gray labware holder, empty 2.2ml, Square-Well Deep Well Plate ("Wash Plate")
Position P3	Gray labware holder, frame for reservoir, reservoirs with reagents (see Figure 2 for configuration)
Position P4	Gray labware holder, MagnaBot® Flat Top Magnetic Separation Device
Position P5	Empty
Position P7	Shaker Integration Plate, VARIOMAG® Teleshake

5.D. Biomek® 3000 Reagent Dispense Volumes

Prior to beginning the run, dispense Automated Differex™ System reagents in the following configuration:

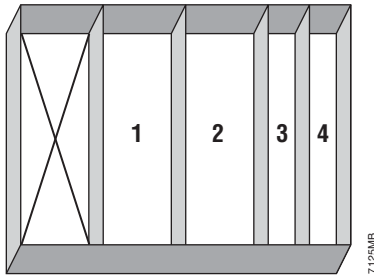


Figure 2. Configuration of troughs at position P3. Troughs 1 and 2 use Quarter Reservoirs. Troughs 3 and 4 use one Quarter Reservoir, Divided by Length.

- | | |
|----------|---|
| Trough 1 | Nuclease-Free Water (if processing more than 24 samples).
Fill with 1,600µl per sample (total samples over 24) plus 2ml dead volume (e.g., for 40 samples, use $40 - 24 = 16$ samples \times 1,600µl = 25.6ml + 2ml dead volume = 27.6ml fill volume). |
| Trough 2 | Nuclease-Free Water (up to 24 samples).
Fill with 1,600µl per sample plus 2ml dead volume (e.g., for 40 samples, use 24 samples total \times 1,600µl = 38.4ml + 2ml dead volume = 40.4ml fill volume). |
| Trough 3 | Resin Solution.
Fill with 100µl per sample plus 1.5ml dead volume (e.g., for 40 samples use $40 \times 100 = 4$ ml + 1.5ml dead volume = 5.5ml total). See Section 4.A for solution preparation. |
| Trough 4 | Separation Solution.
Fill with 125µl per sample plus 2ml dead volume (e.g., for 40 samples, use $40 \times 125\mu\text{l} = 5$ ml + 2ml dead volume = 7ml fill volume). |

6. Description of the Automated Differex™ System Method

This overview describes the general liquid-handling steps required for the Automated Differex™ System and can be adapted to a variety of automated liquid-handling robots. For additional information for adaptation to liquid-handling robots other than those referenced above, see Section 7, General Guidelines for Adaptation to Alternative Robotic Platforms.

1. **Wash Plate Preparation.** Transfer 125µl of Separation Solution from a reservoir to every well of the 96-well, deep-well Wash Plate (position P2) for each column of samples to be processed (half the plate). Transfer 1,600µl of Nuclease-Free Water from a reservoir to each well of the 96-well, deep-well Wash Plate for every column of samples to be processed (second half of the plate). During this step, the Slicprep™ 96 Device can be centrifuged. After preparing the Wash Plate, pause the automated method and place the Sample Plate on the Teleshake position.
2. **Pellet Capping.** Thoroughly resuspend the Resin Solution. Transfer 50µl of Resin Solution to each well of empty, even-numbered columns of the Sample Plate. Cap the sperm pellet by slowly adding 25µl of Resin Solution to each well containing samples. Move plate to the MagnaBot® Flat Top Magnetic Separation Device.
3. **Removal of Epithelial Fraction.** Transfer 75µl of epithelial fraction from each sample well to the adjacent well in the next column. Transfer most of the remaining epithelial fraction volume to the Archival/Waste Plate, leaving a depth of ~2.5mm in the bottom of the sample well. If desired, pause the method to remove and save the Archival/Waste Plate. If the Archival/Waste Plate is removed, it should be replaced with an empty 2.2ml, Square-Well Deep Well Plate to be used for waste. Otherwise, the Archival/Waste Plate will be used as a waste plate.
4. **Wash #1.** Dispense 400µl of Nuclease-Free Water to each sperm-fraction-containing sample well of the Sample Plate. Remove the wash to the Waste Plate, leaving liquid to a depth of ~2.5mm in each sample well.
5. **Wash #2.** Repeat Step 4 for the second wash. Remove the plate from the MagnaBot® Flat Top Magnetic Separation Device.
6. **Wash #3 Addition.** Rapidly add 400µl of Nuclease-Free Water to each sample well to disrupt the sperm pellet. Shake plate to resuspend sperm pellet and release any trapped epithelial material. Pause the method to centrifuge the Sample Plate for 10 minutes at 1,500 × *g*.
7. **Pellet Capping.** Place the Sample Plate onto the MagnaBot® Flat Top Magnetic Separation Device. Thoroughly resuspend the Resin Solution. Slowly add 25µl of Resin Solution to each sample well to cap the sperm pellet.
8. **Underlying of Separation Solution.** Slowly add 115µl of Separation Solution just above the sperm pellet. This will float remaining epithelial material and allow you to remove as much epithelial solution as possible.
9. **Wash #3 Removal.** Remove the Nuclease-Free Water added in Step 6 from the sample wells, leaving the Separation Solution layer intact.
10. **Wash #4.** Dispense 400µl of Nuclease-Free Water to each sample-containing well of the Sample Plate. Remove the wash and Separation Solution to the Waste Plate, leaving liquid to a depth of ~2.5mm in the bottom of the well.

Note: The Archive/Waste Plate can be stored at 4°C for up to 24 hours.

6. Description of the Automated Differex™ System Method (continued)

11. **Method Ends.** The Automated Differex™ System method is now complete, and the original samples have been separated into epithelial and sperm fractions. The separated samples now can either be processed by a DNA isolation method (e.g., DNA IQ™ System) or may be stored at 4°C for up to 24 hours.

Note: The sperm cells are still intact at the end of the method.

7. General Guidelines for Adaptation to Alternative Robotic Platforms

The Resin Solution used for this purification settles rapidly. Ensure that the resin is completely resuspended in the trough before dispensing into processing plates.

The Aspiration and Dispense speeds as well as pipetting heights are critical to the success of this method. Wash solution removal is performed at 66µl/second for the first two aspirations and 10µl/second for each additional aspiration. To dispense 400µl of Nuclease-Free Water (for washes 1, 2 and 4), the addition speeds for each 100µl of liquid are 5µl/second, 10µl/second, 25µl/second and 66µl/second, respectively. The dispensing speed for wash #3 is 100µl/second. All wash dispensing is performed above the level of the liquid in the well. Add Separation Solution at 5µl/second and 3mm above the sperm pellet.

8. Automated DNA IQ™ After Differex™ System Method for the Beckman Coulter Biomek® 3000

8.A. Description

This section describes the Automated DNA IQ™ After Differex™ System Method on the Beckman Coulter Biomek® 3000 Laboratory Automation Workstation. This automated method directly follows the use of the Automated Differex™ System on the Biomek® 3000 (Sections 1–7). We recommend using this DNA IQ™ After Differex™ System method to obtain optimal results in downstream applications (e.g., qPCR or STR amplification). To obtain information about methods for human identification applications, visit: www.promega.com/applications/hmid/automation/

8.B. Materials to be Supplied by the User

- DNA IQ™ System (Cat.# DC6701, DC6700)
- 2.2ml, Square-Well Deep-Well Plate containing Differex™ samples (1; Cat.# V6781)
- 1.2ml Round-Well Deep-Well Plates (1; Cat.# V6771)
- 96-well PCR plate or strip tubes in plate holder or base (1)
- 99% isopropyl alcohol
- 95–100% ethanol
- DTT (Cat.# V3151, V3155)

8.C. Preparation of Solutions

Prior to beginning the Automated DNA IQ™ After Differex™ System Method, prepare the following solutions:

DNA IQ™ Lysis Buffer

Add 6µl of 1M DTT for every 100µl of Lysis Buffer. Mix by inverting several times. Mark and date label to record addition of DTT. This solution can be stored at room temperature for up to one month if sealed, or alternatively, Lysis Buffer plus DTT can be made fresh for each run.

DNA IQ™ 1X Wash Buffer

Add ethanol and isopropyl alcohol directly to the 2X Wash Buffer as directed on the bottle. Replace the cap, and mix by inversion several times. Mark label as 1X Wash Buffer to indicate addition of alcohols. Store at room temperature.

8.D. Beckman Coulter Products Required for the Automated DNA IQ™ After Differex™ System Method on the Biomek® 3000

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required for the Automated DNA IQ™ After Differex™ System method on a Biomek® 3000.

Part Description	Quantity	Beckman Coulter Part Number
Biomek® 3000 Workstation, 50/60Hz, 100–240V	1	986120
Biomek® 3000 Automation Controller XP and Monitor with Biomek® System Software	1	A16170
Biomek® 3000 Left Side Module	1	987264
Biomek® 3000 Right Side Module	1	987263
Gripper Tool Kit for Biomek® 3000	1	A09053
MP200 Eight-Tip Tool	1	986146
Tip Rack Holder	1–4	391910
Gray Labware Holder	6	609120
Frame for Reservoirs	1	372795
Quarter Reservoir	2	372790
Quarter Reservoir, Divided by Length	1	372788
AP96 P250 Barrier Tips	1–4	717253

8.E. Promega Products Required for the Automated DNA IQ™ After Differex™ System Method on the Biomek® 3000

Part Description	Quantity	Promega Cat.#
MagnaBot® 96 Magnetic Separation Device	1	V8151
¼" Foam Spacer	1	Z3301
2.2ml, Square-Well Deep Well Plate (containing samples)	1	V6781
1.2ml, Round-Well Deep Well Plate	1	V6771
Shaker Integration Plate	1	V3691
VARIOMAG® Teleshake 110V, For North American Use Only)	1	V6751
V&P Scientific Heater	1	V6761
Deep Well Heat Transfer Block	1	V6741
96-well plate or strip tubes on plate stand	1	Customer-selected

8.F. Biomek® 3000 Initial Deck Configuration

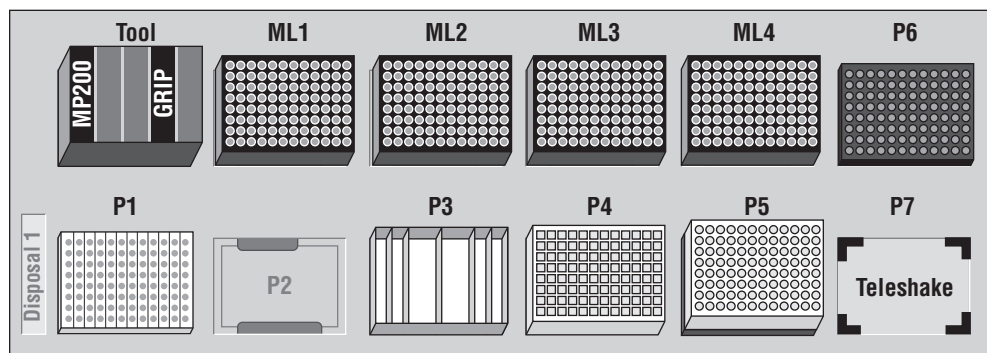


Figure 3. Biomek® 3000 initial deck configuration.

Position Tool	Tool rack containing MP200 and Gripper tools
Position ML1	Tip rack holder, P250 tips
Position ML2	Tip rack holder, P250 tips (include when processing up to 48 samples)
Position ML3	Tip rack holder, P250 tips (include when processing up to 88 samples)
Position ML4	Tip rack holder, P250 tips (include when processing a full plate)
Position P6	Gray labware holder, V&P Scientific Heater, Deep Well Heat Transfer Block
Position P1	Gray labware holder, 96-well PCR plate or strip tubes on plate stand (Elution Plate)
Position P2	Gray labware holder
Position P3	Gray labware holder, Frame for reservoirs, Reservoir with reagents (see Figure 4 for configuration)
Position P4	Gray labware holder, 2.2ml, Square-Well Deep-Well Plate containing Differex™ samples (Sample Plate)
Position P5	Gray labware holder, MagnaBot® Magnetic Separation Device with 1/4" Foam Spacer, Empty 1.2ml, Round-Well Deep-Well Plate (Working Plate)
Position P7	Shaker Integration Plate, VARIOMAG® Teleshake

8.G. Biomek® 3000 Reagent Dispense Volumes

Prior to beginning the run, dispense Automated DNA IQ™ After Differex™ System reagents in the following configuration:

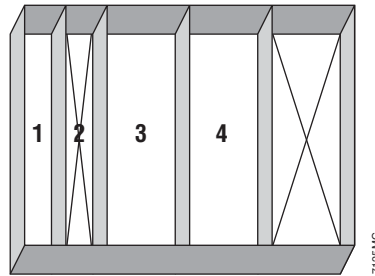


Figure 4. Configuration of troughs at position P3. Trough 1 uses a Quarter Reservoir, Divided by Length. Troughs 3 and 4 each use Quarter Reservoirs.

- Trough 1 DNA IQ™ Elution Buffer
Volume of DNA IQ™ Elution Buffer = 1,200µl + (# of samples × 40µl)
For 96 samples = 1,200µl + (96 × 40µl) = 5,040µl = 5.04ml
- Trough 2 Empty
- Trough 3 DNA IQ™ 1X Wash Buffer (with ethanol and isopropyl alcohol added)
Volume of DNA IQ™ Wash Buffer = 1,500µl + (# of samples × 300µl)
For 96 samples = 1,500µl + (96 × 300µl) = 30,300µl = 30.3ml
(See Section 10.C for solution preparation.)
- Trough 4 DNA IQ™ Lysis Buffer containing DTT (6µl of 1M DTT per 100µl Lysis Buffer)
Volume of DNA IQ™ Lysis Buffer = 1,500µl + (# of samples × 350µl)
For 96 samples = 1,500µl + (96 × 350µl) = 35,100µl = 35.1ml
(See Section 8.C for solution preparation.)

! At the beginning of the automated method, there are a series of user prompts that provide the minimum required volume of each solution per trough.

Number of Samples	DNA IQ™ Elution Buffer*	DNA IQ™ 1X Wash Buffer	DNA IQ™ Lysis Buffer containing DTT
96	10.8ml	30.3ml	35.1ml
80	9.2ml	25.5ml	29.5ml
64	7.6ml	20.7ml	23.9ml
48	6ml	15.9ml	18.3ml
32	4.4ml	11.1ml	12.7ml
16	2.8ml	6.3ml	7.1ml

*These Elution Buffer volumes are specifically calculated for 100µl of elution buffer.

8.H. Description of the Automated DNA IQ™ After Differex™ System Method

This overview describes the general liquid-handling steps required for the Automated DNA IQ™ After Differex™ System Method.

1. **Lysis Buffer Addition.** Add 250µl of Lysis Buffer containing DTT (6µl of 1M DTT per 100µl Lysis Buffer) to each sample well in the Sample Plate.
2. **DNA Binding.** Place the Sample Plate on the shaker. The robot performs a series of shaking and incubation steps to bind DNA to the DNA IQ™ Resin.
3. **Volume Transfer.** Transfer the contents of the Sample Plate to the Working Plate atop the MagnaBot® 96 Magnetic Separation Device, which collects the resin to the sides of each well.
4. **Lysis Buffer Removal.** Remove the supernatant (Lysis Buffer) to the Sample Plate, which now serves as a waste plate.
5. **Lysis Buffer Wash.** Add a second aliquot of 100µl of Lysis Buffer containing DTT to each sample well of the Working Plate to wash the DNA IQ™ Resin. Place the plate on the shaker to mix the resin and buffer.
6. **Lysis Buffer Wash Removal.** Move the Working Plate back onto the MagnaBot® 96 Magnetic Separation Device, and remove the supernatant (Lysis Buffer) to the Waste Plate.
7. **Wash Buffer Addition #1.** Add of 100µl of 1X Wash Buffer containing alcohols to each sample well of the Working Plate. The plate is placed on the shaker to mix the resin and wash buffer.
8. **Wash Buffer #1 Removal.** Move the Working Plate onto the MagnaBot® 96 Magnetic Separation Device, and remove the supernatant (1X Wash Buffer) to the Waste Plate.
9. **Washes #2 and #3.** Repeat Steps 7 and 8 twice for a total of three wash steps.
10. **Drying.** Pause the system for 5 minutes to allow evaporation of any remaining Wash Buffer in the sample wells.
11. **Elution Buffer Addition.** Add a user-defined volume of Elution Buffer to each sample in the Working Plate (e.g., 100µl per well). Place the Working Plate on the shaker, and perform a series of three 30-second shakes and two 2.5-minute heated incubation steps to elute DNA from the DNA IQ™ Resin.
12. **Elution.** Move the Working Plate onto the MagnaBot® 96 Magnetic Separation Device, and remove the supernatant (Elution Buffer containing purified DNA) to the Elution Plate.
13. **Method Ends.** The Automated DNA IQ™ After Differex™ System method is now complete, and the purified DNA samples in the Elution Plate can be processed immediately or stored at 4°C.

8.I. Important Reminders

It is critical to run the Automated DNA IQ™ After Differex™ System method after the Automated Differex™ System method for two reasons:

1. This method uses a sixfold greater amount of DTT in the Lysis Buffer to properly lyse the sperm cells for purification (i.e., 6µl of 1M DTT per 100µl of Lysis Buffer). Other versions of the DNA IQ™ System method only use 1µl of 1M DTT per 100µl of Lysis Buffer, which is insufficient to break open the sperm cells.
2. This method does not add more DNA IQ™ Resin to the samples as the wells already contain a sufficient amount of DNA IQ™ Resin carried over from the Automated Differex™ System method.



Be sure to add DTT to the Lysis Buffer at a ratio of 6µl of 1M DTT per 100µl of Lysis Buffer when using the Automated DNA IQ™ After Differex™ System method.

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Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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