

Automated Differex™ System Protocol for the Tecan Freedom EVO® System

Automated Protocol #EP030

DESCRIPTION OF THE TECAN FREEDOM EVO® PROGRAM FOR THE AUTOMATED 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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I. Description

This document describes the Differex™ System^(a) on the Tecan Freedom EVO® automated liquid-handling workstation. To obtain information about methods for human identification applications, visit:

www.promega.com/applications/hmid/automation/. General automation guidelines are provided for adaptation to other liquid-handling platforms in Section VII. For troubleshooting chemistry issues, please refer to the *Differex™ System Technical Bulletin #TBD020*.

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

II. Product Requirements and Storage Conditions

Note: The Differex™ System was initially developed for use in a manual format. Reagent volumes have been adjusted for the automated method. However, the Differex™ System kits (Cat.# DC6801, DC6800) will not provide the listed number of sample isolations in the automated format. We recommend the reagents be purchased as standalone products.

Product	Size	Cat.#
Differex™ Digestion Buffer*	150ml	A8501
Differex™ Separation Solution*	40ml	A8511
Nuclease-Free Water**	50ml	P1193
	150ml	P1195

*Not for Medical Diagnostic Use.

**For Laboratory Use.

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

Items Available Separately

Product	Size	Cat.#
Slicprep™ 96 Device*	10 pack	V1391
Pyramid-Bottom Reservoir, 12 Column	25/case	V6791
2.2ml, Square-Well Deep Well Plate	50/case	V6781
MagnaBot® Flat Top Magnetic Separation Device	each	V6041
Proteinase K*	100mg	V3021
DTT	5g	V3151
	25g	V3155
DNA IQ™ System*	100 reactions	DC6701
	400 reactions	DC6700
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*	A8251	1 bottle (50ml)

*DNA IQ™ Resin can also be obtained from the 400 reaction DNA IQ™ System (Cat.# DC6700).

III. Materials to be Supplied by the User

- Centrifuge capable of $1,500 \times g$, 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/profile.asp?appname=Genetic+Identity&sku=V1391&spl=off and select the “Centrifuge Compatibility” tab)
- 2.2ml, Square-Well Deep Well Plates (2; Cat.# V6781)
- Pyramid-Bottom Reservoir, 12 Column (Cat.# V6791)
- Slicprep™ 96 Device (Cat.# V1391)
- MagnaBot® Flat Top Magnetic Separation Device (Cat.# V6041)
- 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])

IV. Before You Begin

IV.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.

Digestion Solution

Digestion Solution should be prepared 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 $40 \times 400\mu\text{l} = 16\text{ml}$ Digestion Solution by mixing $40 \times 25\mu\text{l} = 1\text{ml}$ Proteinase K Solution with $40 \times 375\mu\text{l} = 15\text{ml}$ Digestion Buffer. Include an additional 75µl of Proteinase K and 1,125µl of Digestion Buffer for the needed dead volume.

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:

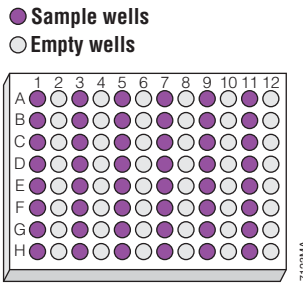
Resin volume = (#samples \times 14µl of DNA IQ™ Resin) + 210µl dead volume
Nuclease-Free Water volume = (#samples \times 86µl) + 1,290µl dead volume

For example, if there are 40 samples:

Resin volume = $(40 \times 14\mu\text{l of DNA IQ}^{\text{TM}} \text{ Resin}) + 210\mu\text{l dead volume} = 770\mu\text{l}$
Nuclease-Free Water volume = $(40 \times 86\mu\text{l}) + 1,290\mu\text{l dead volume} = 4,730\mu\text{l}$

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

IV.B. Sample Preparation Before Automated Processing



Prepare a Slicprep™ 96 Device by removing the spacer and pushing the device into the deep-well plate. Sexual assault swabs should be placed in columns 1, 3, 5, 7, 9 and 11 of a Slicprep™ 96 Device. To each sample, add 400µl of Digestion Solution and then seal the top of the device with a plate sealer. Place the Slicprep™ 96 Device in a water bath or incubator at 56°C for 1.5 hours. After digestion, raise the top of the Slicprep™ 96 Device and insert the spacer between the plate and filter portion. Start the automated method while spinning the Slicprep™ 96 Device at 1,500 × g for 10 minutes.

V. Automated Processing Requirements for the Tecan Freedom EVO® Workstation

This section lists the instrument and labware requirements for the Automated Differex™ System method on the Tecan Freedom EVO® Workstation.

V.A. Instrumentation Requirements for Automated Differex™ System on the Tecan Freedom EVO® Workstation

The following is a list of Tecan parts and their corresponding part numbers that are required for the Automated Differex™ System method on a Freedom EVO® Workstation.

Part Description	Quantity	Tecan Part Number
Tecan Freedom EVO® configured for the DNA IQ™ System	1	Tecan Package Sheet #1006
Trough, Disposable, 100ml, Polypropylene Natural	2	10613049
Microplate Carrier, 3 Position, Landscape	1	10612604

V.B. Labware Requirements for Automated Differex™ System on the Tecan Freedom EVO® Workstation

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
Pyramid-Bottom Reservoir, 12 Column	1	V6791

V.C. Tecan Freedom EVO® Initial Deck Configuration

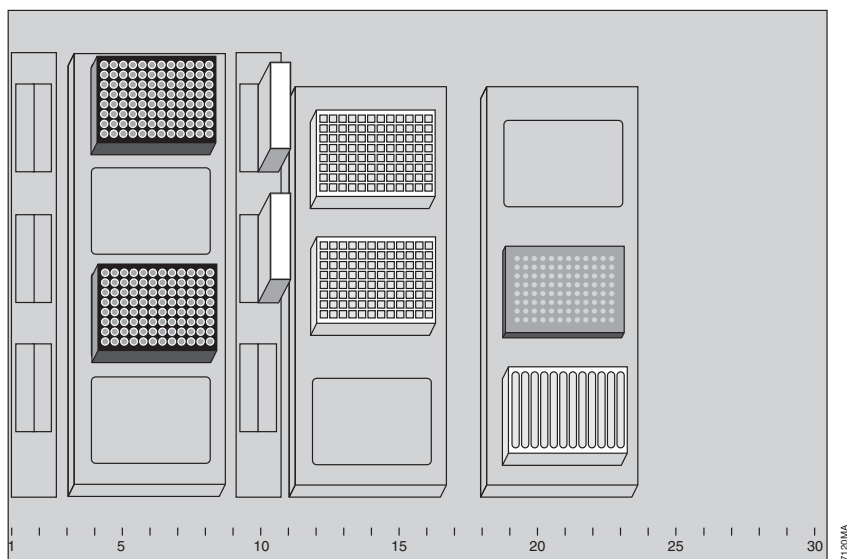


Figure 1. Tecan Freedom EVO® initial deck configuration.

Grid 1	Trough Holder with associated tip rack positions	
	Position 1 (rear)	Empty
	Position 2 (middle)	Empty
	Position 3 (front)	Empty
Grid 3	4-Position Tip Rack Holder	
	Position 1 (rear)	1ml tips
	Position 2 (mid rear)	Empty
	Position 3 (mid front)	200µl tips
	Position 4 (front)	Empty
Grid 5	12-position Shelf	Empty
	(back of deck)	
Grid 9	Wash Station with tip disposal and associated trough rack	
	Position 1 (rear)	100ml trough
	Position 2 (middle)	100ml trough
	Position 3 (front)	Empty
Grid 12	3-position microplate holder	
	Position 1 (rear)	2.2ml, Square-Well Deep Well Plate, Empty
	Position 2 (middle)	2.2ml, Square-Well Deep Well Plate, Empty
	Position 3 (front, shaker)	Empty
Grid 18	2-position microplate holder with Te-Shake™	
	Position 1 (rear)	Empty
	Position 2 (middle)	MagnaBot® Flat Top Magnetic Separation Device
	Position 3 (front)	Pyramid-Bottom Reservoir, 12 Column
Grid 25	VWR Heater	Empty
	(back of deck)	

V.D. Tecan Freedom EVO® Reagent Dispense Volumes

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

Grid 9 Position 1 (rear) = **Separation Solution**

Fill with 125µl per sample plus 2ml dead volume (e.g., to calculate for 40 samples, use $40 \times 135\mu\text{l} = 5.4\text{ml} + 2\text{ml}$ dead volume = 7.4ml fill volume).

Position 2 (middle) = **Nuclease-Free Water**

Fill with 1,600µl per sample plus 2ml dead volume (e.g., to calculate for 40 samples, use $40 \text{ samples} \times 1,600\mu\text{l} = 64\text{ml} + 2\text{ml}$ dead volume = 66ml fill volume).

Grid 18 Position 3 (front in column 2 of Pyramid-Bottom Reservoir, 12 Column) = **Resin Solution**

Fill with 100µl per sample plus 1.5ml dead volume (e.g., for 40 samples use $40 \times 100 = 4\text{ml} + 1.5\text{ml}$ dead volume = 5.5ml total). See Section IV.A for solution composition.

VI. 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 VII, General Guidelines for Adaptation to Alternative Robotic Platforms.

1. **Wash Plate Preparation.** Transfer 125µl of Separation Solution from a reservoir to the 96-well, deep-well Wash Plate for each column of samples to be processed. Then transfer 1,600µl of Nuclease-Free Water from a reservoir to the 96-well, deep-well Wash Plate for each column of samples to be processed. During this step, the Slicprep™ 96 Device can be spun. After preparing the wash plate, pause the automated method to place the Sample Plate on the instrument.
2. **Pellet Capping.** Thoroughly resuspend the Resin Solution. Transfer 50µl of Resin Solution to empty, even-numbered columns. Cap the sperm pellet by slowly adding 25µl of Resin Solution to the wells containing samples. Move plate to the MagnaBot® Flat Top Magnetic Separation Device.
3. **Removal of Epithelial Fraction.** Transfer 75µl of epithelial fraction from the sample wells to the adjacent well in the next column. Transfer most of the remaining epithelial fraction volume to the Archival Plate, leaving a depth of ~2.5mm in the bottom of the sample well. If desired, pause the method to remove the Archival Plate. If the Archival 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 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 the sample well to disrupt the sperm pellet and release any trapped epithelial material. Then pause the method to centrifuge the sample plate for 10 minutes at 1,500 × g.
7. **Pellet Capping.** Place the plate onto the MagnaBot® Flat Top Magnetic Separation Device. Thoroughly resuspend the Resin Solution. Slowly add 25µl of Resin Solution to the sample wells to cap the sperm pellet.
8. **Underlaying of Separation Solution.** Slowly add 115µl of Separation Solution just over the sperm pellet. This will float remaining epithelial material and make it easier to remove.
9. **Wash #3 Removal.** Remove the wash 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.
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 can now either be processed by a DNA isolation method (e.g., DNA IQ™ System) or may be stored at 4°C.

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

VII. 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 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. 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.

(a) Patents Pending.

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