

# TECH TIPS

## Automating the Differex™ System

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### How can I automate the Differex™ System?

The automated Differex™ protocol combines two automated methods and two reagent systems, the Differex™ System<sup>(k)</sup> (Cat.# DC6800) and the DNA IQ™ System (Cat.# DC6700), to perform differential extraction and DNA purification from sexual assault samples. The first automated method involves differential extraction of sexual assault samples into epithelial and sperm fractions. In the second automated method, genomic DNA is purified from each fraction.

### How does the automated Differex™ system work?

The automated Differex™ protocol begins with a proteinase K digestion of samples in a Slicprep™ 96 Device. Samples are then centrifuged to separate digestion products from the solid support material and pellet sperm. The resultant plate with sperm pellets and supernatants containing the epithelial DNA is placed onto the deck of a robotic workstation, and the automated Differex™ method begins.

The automated Differex™ method allows high-throughput, automated differential extraction of sexual assault samples by combining the Differex™ System and DNA IQ™ Resin in a novel pellet-capping process. First, the robot dispenses DNA IQ™ Resin directly on

top of each sperm pellet. Upon application of a magnetic field, the paramagnetic resin particles form a capping layer, which allows supernatant manipulation without pellet disruption.

A portion of the supernatant is then removed from each sample well and moved to an adjacent well for retention and downstream processing as the epithelial fraction. The remainder of each supernatant is removed from the pellet and discarded or, if desired, archived. Resin-capped sperm pellets are then washed four times to dilute and remove the epithelial DNA-containing supernatant. During the third wash, the resin-capped sperm pellets are resuspended to release any trapped epithelial material. Resuspending the pellets requires a manual step to centrifuge the sample plate and pellet the sperm. Following this centrifugation the robot adds a second aliquot of DNA IQ™ Resin to re-establish the pellet cap. The Differex™ Separation Solution is used to float the residual epithelial DNA-containing wash buffer away from the capped pellet to further improve epithelial DNA removal. A fourth and final wash is performed to complete the automated Differex™ extraction. All sperm and epithelial fractions then undergo automated DNA isolation using the DNA IQ™ System.

### How does the automated Differex™ separation differ from traditional differential extraction?

Many aspects of the automated Differex™ method are familiar to analysts who have used a traditional differential extraction protocol. Both processes use proteinase K digestion to selectively lyse epithelial cells, a centrifugation to free digestion products from the solid support and pellet sperm, and a series of pellet washes to dilute digestion products in the supernatant.

The difference with the automated Differex™ protocol is its pellet-capping process. DNA IQ™ Resin forms a pellet-capping layer on top of the sperm pellet. This paramagnetic pellet restraint allows the epithelial DNA-containing supernatant to be washed away without the need to recentrifuge samples after every wash. The Differex™ Separation Solution is then used to float residual wash buffer away from sperm pellets, allowing easy removal from what will become the sperm fraction.

Automated Differex™ extraction reproducibly automates the process of differential extraction, allowing an unprecedented level of throughput in processing sexual assault samples. Additionally, incorporation of DNA IQ™ System components into the automated Differex™ protocol allows a high-throughput DNA isolation alternative to laboratories that are still performing organic nucleic acid isolation.

**How does automated Differex™ separation differ from the manual Differex™ protocol?**

The automated and manual Differex™ technologies differ in how reagents are used to separate sperm from epithelial DNA after proteinase K digestion. The manual technology uses a spin-through process where sperm are centrifuged through a layer of Separation Solution to physically separate sperm from epithelial cell digestion products. The automated Differex™ protocol applies a pellet-capping process, where the DNA IQ™ Resin protects the sperm pellet during washing, and the Differex™ Separation Solution helps remove the epithelial DNA-containing supernatant from the particulate sperm pellet. Integration of DNA IQ™ and Differex™ System components results in higher sperm fraction yields and reproducible, automated sexual assault sample processing.

**Which platforms are compatible with the automated Differex™ protocol?**

Automated Differex™ methods are available for the Beckman Coulter Biomek® 2000 and Biomek® 3000 automated workstations as well as the Tecan Freedom EVO® platform.

**What throughput does the automated Differex™ protocol provide?**

Because each sample is split into two fractions, a maximum of 48 samples can be processed at one time using the automated Differex™ protocol (40 samples can be processed if wells

are reserved for DNA standards in downstream DNA quantitation). The Beckman Coulter Biomek® 2000 and Biomek® 3000 workstations allow automated Differex™ differential extraction and DNA purification in eight-sample increments. On the Tecan Freedom EVO® platform, any number of samples can be processed. Process times are shown in Table 1.

**What laboratory resources do I need to perform automated Differex™ separation?**

To perform automated Differex™ separation, you will need the following:

- Tecan Freedom EVO®, Beckman Coulter Biomek® 2000 or Biomek® 3000 automated workstation configured for use of the DNA IQ™ System
- MagnaBot® Flat Top Magnetic Separation Device (Cat.# V6041)
- Slicprep™ 96 Device (Cat.# V1391) for preprocessing sexual assault samples
- 2.2ml, Square-Well Deep Well Plate (Cat.# V6781)
- Proteinase K (Cat.# V3021) and DTT (Cat.# V3151)
- DNA IQ™ System (Cat.# DC6700)

- A swinging-bucket rotor that can centrifuge the Slicprep™ 96 Device at  $\geq 1,500 \times g$ . See a list of suitable centrifuges at: [www.promega.com/slicprepcentrifuges/](http://www.promega.com/slicprepcentrifuges/)
- A 56 °C water bath or oven

**How will the automated Differex™ protocol integrate with existing Promega technologies (or into laboratory workflow)?**

We designed the automated Differex™ method as a starting point to integrate the complete line of Promega nucleic acid isolation, quantification, STR amplification and analysis technologies. The automated Differex™ method incorporates DNA IQ™ chemistry to purify DNA from differential extraction samples. Male-specific and total human autosomal DNA from sperm and epithelial fraction preparations can be quantified using the Plexor® HY System and amplified using the PowerPlex® 16 System. (For Biomek® 2000 users, Plexor® HY results can be imported directly into the PCR Normalization Wizard for automated setup of PowerPlex® 16 reactions). Capillary electrophoresis results for each fraction can then be analyzed using the FSS-i3™ Expert Systems Software.

**Table 1. Processing Times for the Automated Differex™ Method.**

Step	Processing Time for One Column (8 Samples)	Processing Time for Five Columns (40 Samples)
Initial proteinase K digestion	90 minutes	90 minutes
Automated Differex™ method		
Centrifugation	10 minutes	10 minutes
Washes 1 and 2 of the automated Differex™ method	25 minutes	50 minutes
Centrifugation	10 minutes	10 minutes
Washes 3 and 4 of the automated Differex™ method	20 minutes	45 minutes
DNA purification using the DNA IQ™ System	45 minutes	95 minutes
<b>Total time<sup>1</sup></b>	<b>190 minutes</b>	<b>290 minutes</b>

<sup>1</sup>Hands-on time is much less. Total time does not include the first centrifugation step, since this centrifugation occurs at the same time as the first few steps of the automated Differex™ method.