TECH TIPS

The Differex™ System

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The Differex™ System[d] (Cat.# DC6800 and DC6801) is a new, improved method for separating sperm and epithelial fractions in sexual assault samples. Following a standard proteinase K digestion, the sperm pellet is separated from the epithelial fraction using the unique Separation Solution (Figure 1), which allows rapid fractionation with only one centrifugation. In combination with the DNA IQ™ System, the Differex™ System provides a simple, rapid purification system that takes the art out of differential extraction.

Figure 1. Separation of the sperm pellet and epithelial DNA. Following a proteinase K digestion to lyse epithelial cells, the sample and Digestion Buffer are placed in a DNA IQ™ Spin Basket seated in a tube containing the Separation Solution. During a single centrifugation, the sperm collect at the bottom of the tube, separated from the epithelial DNA, which remains in the Digestion Buffer.

What is required to use the Differex™ System?

In addition to the Differex™ System, the following are required:

- Proteinase K (10–20mg/ml) (Cat.# V3021)
- barrier pipette tips
- Microcentrifuge Tubes (Cat.# V1231)
- DNA IQ™ Spin Baskets (Cat.# V1221)
- microcentrifuge
- 56°C or 37°C heat block or oven

To isolate DNA from the epithelial and sperm fractions obtained using the Differex™ System, a DNA purification system, such as the DNA IQ™ System, is also required.

How long does it take to perform the differential extraction? How long to isolate DNA?

Differential extraction of 10 samples can be completed in 90 to 100 minutes using the Differex™ System. The hands-on time is only 30 to 40 minutes with a 1-hour proteinase K digestion at 56°C. Differential extraction followed by manual DNA isolation from both the epithelial and sperm fractions using the DNA IQ™ System requires a total of approximately 3 hours for 10 samples.

At what temperatures can I perform the proteinase K digestion?

We have successfully performed the proteinase K digestion at 37°C for 2 hours and 56°C for 1 hour. In most cases, this is sufficient to lyse all epithelial cells.

Which DNA purification protocols have been used with the Differex™ System?

We have tested and validated the Differex™ System with the DNA IQ™ System. DNA purification by organic extraction has also been successful with epithelial and sperm fractions obtained using the Differex™ System. Other protocols are being tested. Contact Promega Technical Services at: genetic@promega.com for information regarding other DNA purification methods.

Can the Differex™ System be automated?

We are developing protocols and an apparatus to allow differential extraction using the Differex™ System on robotic liquid handlers. Contact your local Promega representative for more information.

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Can the sperm fraction be analyzed microscopically prior to DNA isolation?

Yes. However, only the sperm heads are detected at this stage since the sperm tails will be digested during the initial proteinase K digestion (Figure 2).

To microscopically examine your sperm pellet, use a pipette to remove as much of the Separation Solution as possible without disturbing the pellet. Add 50–100µl of TE^{-4} buffer and mix by vortexing to resuspend the pellet. Spot 5–10µl of the resuspension on a microscope slide. Follow your laboratory's procedure for the staining and microscopic detection of sperm cells.

Users of the Differex™ System should not attempt to remove a portion of the sperm pellet without first removing the Separation Solution. Vortex mixing in the Separation Solution will not adequately resuspend the pellet, resulting in an inaccurate estimate of the number of cells present in the sperm fraction.

I see residual Digestion Solution at the top of my tube. How can I remove it?

Digestion Solution on the sides of the tube will be removed by the water wash step. If there is Digestion Solution in the cap, remove it with a Kimwipes® tissue or centrifuge the tube briefly to force the liquid to the bottom of the tube.

Figure 2. Microscopic analysis of sperm. Following differential extraction using the Differex™ System, the Separation Solution was removed from the sperm pellet, and 100µl of TE^{-4} buffer was added. Cells were resuspended by vortex mixing, stained then analyzed microscopically. Sperm heads are indicated by the arrows.

After performing the differential extraction, I observe a large clump of what appears to be cellular material in the Separation Solution. What is this material?

There are two main sources for this material. A large tight cell pellet can be caused by incomplete digestion of epithelial cells. A large diffuse pellet can result from a large amount of mucus in the sample. A longer proteinase K digestion can help (up to 2 hours at 56°C). Diffuse pellets often contain debris with little or no DNA and can usually be removed by carefully pipetting away the solution, leaving the tight sperm pellet behind.

Now that I've performed the separation, can I store my samples prior to purifying the DNA?

We have not investigated the effects of storing the sperm pellet in Separation Solution overnight, so we recommend proceeding with the DNA isolation. If DNA will be purified from the fractions using the DNA IQ™ System, this additional step requires only 30–60 minutes to perform manually, depending on the number of samples.