TECHNICAL MANUAL

Differex™ System

Instructions for Use of Products
DC6800 and DC6801

Revised 6/23
TM331

Differex™ System

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E-mail Promega Technical Services if you have questions on use of this system: genetic@promega.com

1. Description

In 1985, Gill *et al*. (1) developed a method to enrich for sperm cells in the presence of an excess of epithelial cells. After a controlled lysis of the epithelial cells in the absence of a reducing agent, the sample is centrifuged in a spin basket to remove intact sperm and buffer containing the DNA from lysed epithelial cells from the solid matrix. The centrifugation pellets the sperm and cell debris. The solution containing the epithelial DNA is removed, but because of the loose nature of the pellet, some solution containing epithelial DNA remains. To obtain sperm free of epithelial DNA, several washings and recentrifugations are required. Although time-consuming and labor-intensive, the process has remained the method

1. Description (continued)

of choice without any major changes for many decades. This process strikes a balance between epithelial DNA removal and loss of sperm during the washings. Due to the competing nature of removing epithelial DNA and losing sperm, considerable time is spent by examiners to determine how much sample to use and how many times to wash, and so the examiner's level of experience plays an important role in determining the success of the separation process.

The Differex™ System[a] uses a standard proteinase K digestion and a combination of phase separation and differential centrifugation to separate sperm and epithelial DNA. After a standard proteinase K digestion, the sample and buffer are placed in a spin basket and centrifuged to remove the solid support. During centrifugation, the sperm pellets near the bottom of the tube. If desired, DNA IQ™ Resin can be added to the tube opposite the sperm pellet to reduce sperm cell loss during the process. Then the tube is placed on the Differex™ Magnet (Figure 1). The magnet has two different positions because the position and shape of the pellet formed after each centrifugation step differs slightly. The resin will migrate to the magnet and cap the sperm pellet. The two separate magnets allow the DNA IQ™ Resin to form a better cap over the pellets.

The aqueous epithelial DNA-containing fraction is removed while the tube is in the magnet holder. The resin-capped pellet is washed with no centrifugation required. To aid efficient removal of the remaining wash solution, the nonaqueous Separation Solution, which is denser than the wash solution, is added to form a layer between the sperm pellet and residual wash solution. The wash solution then is easily removed. Removal of the aqueous solution is aided by the yellow color of the Digestion Buffer. Only a thin film of aqueous solution usually remains. Any contaminating aqueous solution is easily removed by performing an additional wash with water. The water is not miscible with the Separation Solution and can be easily removed without additional centrifugation. The use of the Separation Solution instead of a membrane eliminates clogging issues, and the nonaqueous nature of the Separation Solution helps to form a tight sperm pellet and keep cell debris away from the sperm pellet.

Figure 1. The Differex™ Magnet.

DNA from sperm and epithelial fractions can be isolated using the manual DNA IQ™ System or automated DNA IQ™ chemistry using a Maxwell® Instrument. See Sections 4–6.

The total time to separate sperm from epithelial cells after adding the sample to the proteinase K-containing Digestion Solution is approximately 2 hours, which includes a 90-minute proteinase K digestion. DNA purification requires 30 minutes, so separation and purification can be accomplished in as little as 2.5 hours. DNA also can be purified from the separated epithelial and sperm fractions using phenol:chloroform-based methods, although more time is required.In addition, the optional step using DNA IQ™ Resin to cap the pellet can result in fewer sperm lost during the process. Finally, the familiar proteinase K digestion from the standard methodology is retained for the upfront processing of the solid support.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Differex™ System	**50 samples**	**DC6801**

Not for Medical Diagnostic Use. Includes enough reagents for 50 samples:

- 25ml Digestion Buffer
- 5ml Separation Solution*
- 2 × 25ml Nuclease-Free Water

PRODUCT	SIZE	CAT.#
Differex™ System	**200 samples**	**DC6800**

Not for Medical Diagnostic Use. Includes enough reagents for 200 samples:

- 100ml Digestion Buffer
- 20ml Separation Solution*
- 2 × 150ml Nuclease-Free Water

Storage Conditions: Store all components at room temperature.

*The Separation Solution is nontoxic and biodegradable and can be used outside of a hood in a room with good airflow.

The Differex™ System, Digestion Buffer and Nuclease-Free Water are manufactured in accordance with ISO 18385:2016.

Available Separately

PRODUCT	SIZE	CAT.#
DNA IQ™ Resin[1]	**8ml**	**A8258**
	50ml	**A8251**
Nuclease-Free Water	**150ml**	**P1196**

[1]Only for use with Section 3.B protocol.

3. Separating Sperm from Epithelial Cells

Follow current guidelines when determining the size of sample to process. Minimize carryover of epithelial DNA into the sperm fraction by using only enough sample to obtain sufficient amounts of sperm for analysis.

Materials to Be Supplied by the User for Differential Extraction

- DNA IQ™ Resin (Cat.# A8251, A8258; only use with Section 3.B protocol)
- Nuclease-Free Water (Cat.# P1196)
- Proteinase K (Cat.# V3021)
- barrier tips
- ClickFit Microtube, 1.5ml (Cat.# V4745)
- DNA IQ™ Spin Baskets (Cat.# V1225)
- microcentrifuge
- heat block or oven at 56°C
- Manual Differex™ Magnet (Cat.# V1591; only use with Section 3.B protocol)

Materials to Be Supplied by the User for DNA Purification Using the DNA IQ™ System

- DNA IQ™ System (Cat.# DC6701 or DC6700)
- ethanol
- isopropyl alcohol
- DTT, Molecular Grade (Dry Powder; Cat.# V3151)
- heat block or oven at 65°C

Materials to Be Supplied by the User for DNA Purification Using the Maxwell® 16 Instrument

- Casework Extraction Kit (Cat.# DC6745)
- DNA IQ™ Casework Pro Kit (Cat.# AS1240)

Materials to Be Supplied by the User for DNA Purification Using the Maxwell® FSC Instrument

- Maxwell® FSC Instrument (Cat.# AS4600)
- Casework Extraction Kit (Cat.# DC6745)
- Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550)

We highly recommend the use of gloves and aerosol-resistant pipette tips. Use the ClickFit Microtube, 1.5ml, to prevent inadvertent opening of caps during heated incubation.

3.A. Preparing Digestion Solution

1. Add proteinase K to the Digestion Buffer to a final concentration of 270µg/ml to prepare the Digestion Solution. Each sample will require 400µl of Digestion Solution.

   Do not store and reuse the Digestion Solution once the proteinase K is added. The concentration of proteinase K can be adjusted for the number of epithelial cells and the quality of the sample based on previous experience with differential lysis.

2. Mix and use immediately.

3.B. Differential Extraction Protocol With the Differex™ Magnet

1. Place the solid support containing sperm in a ClickFit Microtube, 1.5ml.
2. Add 400µl of yellow Digestion Solution prepared in Section 3.A to the sample.
3. Close the tube cap, and vortex at high speed for 30 seconds. Be sure to keep the tube vertical while vortexing. Place the tube at 56°C for 90 minutes.
4. For each sample processed in Step 3, place a DNA IQ™ spin basket into a new, labeled ClickFit Microtube, 1.5ml.
5. After the proteinase K digestion (Step 3), remove the solid support from the Digestion Solution and place it in the spin basket prepared in Step 4. Slowly pipet the remaining Digestion Solution into the spin basket. Some of the solution may flow through the spin basket.
6. Close the cap on the spin basket. Mark the tube where you expect the sperm pellet to form. This will assist in Step 8 should the sperm pellet not be visible. Centrifuge for 10 minutes at maximum speed (14,000rpm) in a microcentrifuge at room temperature. After centrifugation, the tube may contain a small, slightly yellow or white pellet of sperm and a yellow aqueous layer containing epithelial DNA in Digestion Solution.
7. Remove and discard the spin basket. Remove any yellow Digestion Solution from the tube cap with a Kimwipes® tissue or pipette. Alternatively if any Digestion Solution remains in the tube cap, briefly centrifuge to force the contents to the bottom of the tube. To avoid carryover of epithelial DNA into the sperm fraction, be sure that no liquid remains in the cap after the centrifugation.
8. Vortex the stock DNA IQ™ Resin bottle for 10 seconds at high speed or until resin is thoroughly mixed. Add 3.5µl of DNA IQ™ Resin near the bottom of the tube on the side opposite the sperm pellet. Place the tube into the Differex™ Magnet (position 1), being careful to align the pellet (or the marking) with the magnet to allow the resin to cover the pellet. Leave the tube in position 1 for Steps 9 and 10.
9. Remove and reserve as much of the yellow aqueous layer as possible for epithelial DNA purification in Section 4.B, 5.B or 6.B.

   Note: Removing all droplets from the side of the tube is not necessary. The droplets will be removed during the wash steps.

10. Wash the resin-capped pellet by slowly adding 500µl of Nuclease-Free Water, being careful to rinse the tube walls of any residual yellow digestion solution. Remove and discard the water. Repeat this wash step with another 500µl of Nuclease-Free Water.

3.B. Differential Extraction Protocol With the Differex™ Magnet (continued)

11. Add a third wash volume of 500µl Nuclease-Free Water to the sample tube, and vortex sample briefly to resuspend resin particles.
12. Replace tubes into microcentrifuge, and centrifuge for 10 minutes at maximum speed (14,000 rpm) at room temperature.
13. Vortex the stock DNA IQ™ Resin bottle for 10 seconds at high speed or until resin is thoroughly mixed. Following centrifugation, immediately add 7µl of DNA IQ™ Resin near the bottom of tube opposite the new pellet. This pellet will be in a different position than the pellet formed in Step 6 and will also contain the DNA IQ™ Resin. Place spin tubes into Differex™ Magnet (position 2), being careful to align sperm/resin pellet with magnet, and allow the additional resin to cover the pellet. Leave the tube in position 2 of the Differex™ Magnet for Steps 14 and 15.
14. Remove wash solution, and add a fourth 500µl wash volume.
15. Slowly add 100µl of Separation Solution to the side of the tube, and let it settle beneath the aqueous layer so that it covers the resin pellet. Be careful not to disturb the pellet.
16. Remove wash solution and as much Separation Solution as possible without disrupting the pellet. Reserve contents of tube as the sperm fraction.

Notes:

a. For most samples, 100µl of the yellow solution reserved in Section 3.B, Step 9 is sufficient to obtain enough epithelial DNA for genotype analysis. DNA from the entire epithelial fraction can be purified if desired.

b. The yellow dye will not interfere with DNA amplification and will be removed during the purification process.

3.C. Differential Extraction Protocol Without a Magnet

1. Place the solid support containing sperm in a ClickFit Microtube, 1.5ml.
2. Add 400µl of yellow Digestion Solution prepared in Section 3.A to the sample.
3. Close the tube cap, and vortex at high speed for 30 seconds. Be sure to keep the tube vertical while vortexing.Place the tube at 56°C for 90 minutes.
4. For each sample processed in Step 3, place a DNA IQ™ spin basket into a new, labeled ClickFit Microtube, 1.5ml, containing 100µl of Separation Solution.
5. After the proteinase K digestion (Step 3), remove the solid support from the Digestion Solution and place it in the spin basket prepared in Step 4. Slowly pipet the remaining Digestion Solution into the spin basket. Some of the solution may flow through the spin basket.
6. Close the cap on the spin basket. Mark the tube where you expect the sperm pellet to form. This will assist in Step 8 should the sperm pellet not be visible. Centrifuge for 10 minutes at maximum speed (14,000rpm) in a microcentrifuge at room temperature. After centrifugation, the tube may contain a small, slightly yellow or white pellet of sperm and a yellow aqueous layer containing epithelial DNA in Digestion Solution.

7. Remove and discard the spin basket. Remove any yellow Digestion Solution from the tube cap with a Kimwipes® tissue or pipette. Alternatively if any Digestion Solution remains in the tube cap, briefly centrifuge to force the contents to the bottom of the tube. To avoid carryover of epithelial DNA into the sperm fraction, be sure that no liquid remains in the cap after the centrifugation.
8. Remove and reserve as much of the yellow aqueous layer as possible for epithelial DNA purification in Section 4.B, 5.B or 6.B.

   Note: Removing all droplets from the side of the tube is not necessary. The droplets will be removed during the wash steps.
9. Pipet 500µl of Nuclease-Free Water on top of the Separation Solution, washing the sides of the tube to remove any droplets of yellow buffer. Some mixing of the water and Separation Solution will not affect the results, but be careful not to disturb the pellet.
10. Wait 30 seconds or more, then remove and discard the upper water layer, up to a third of the clear Separation Solution and any cell debris near the boundary of the two solutions. This wash step dilutes and removes buffer containing the epithelial DNA at the interface and on the sides of the tube.

    Do not disturb the pellet.

    Do not add the Separation Solution and cell debris to the fraction that will be used to purify epithelial DNA. Be careful not to disturb the small pellet at the bottom of the tube.
11. Perform a second water wash by repeating Steps 9 and 10.

    Note: The color of the first water wash in Step 9 is an indication of the success in removing the yellow layer containing the epithelial DNA. If the first water wash is clear and the sample contains a low number of epithelial cells, a second water wash may not be necessary. Separation is complete.

4. DNA Extraction from Sperm and Epithelial Fractions Using the DNA IQ™ System

See the *DNA IQ™ System—Small Sample Casework Technical Bulletin* #TB296 for information on how to prepare the DNA IQ™ Lysis Buffer with DTT.

Vortex the resin bottle for 10 seconds at high speed or until resin is mixed. Keep the resin resuspended while dispensing to obtain uniform results.

4.A. DNA Extraction from Sperm Fraction Using the DNA IQ™ System

1. To extract DNA from the sperm fraction collected using the Differential Extraction Protocol with the Differex™ Magnet (Section 3.B), add 250µl (or at least 2 volumes) of DNA IQ™ Lysis Buffer containing DTT.
2. To extract DNA from the sperm fraction collected from Differential Extraction Protocol without a magnet (Section 3.C), add 250µl (or at least 2 volumes) of DNA IQ™ Lysis Buffer containing DTT and 7µl of DNA IQ™ Resin.
3. Proceed with DNA purification as described in the *DNA IQ™ System—Small Sample Casework Technical Bulletin* #TB296 (Section 4.F, Steps 4–16).

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4.B. DNA Extraction from Epithelial Fraction Using the DNA IQ™ System

1. Epithelial DNA is purified from the yellow aqueous layer reserved in Section 3.B, Step 9 or Section 3.C, Step 8.
2. Add 2 volumes of DNA IQ™ Lysis Buffer with DTT to the yellow solution and mix.
3. Add 7µl of DNA IQ™ Resin, vortex briefly, and incubate at room temperature for 5 minutes.
4. Proceed with DNA purification as described in the *DNA IQ™ System—Small Sample Casework Technical Bulletin* #TB296 (Section 4.F, Steps 5–16).

5. DNA Extraction from Sperm and Epithelial Fractions Using DNA IQ™ Chemistry on the Maxwell® 16 Instrument

5.A. DNA Extraction from Sperm Fraction Using DNA IQ™ Chemistry and the Maxwell® 16 Instrument

1. To the sperm fraction, add 10µl of Proteinase K solution (18mg/ml), 4µl of 1-Thioglycerol and Casework Extraction Buffer to a total volume of 400µl. Do not exceed a final volume of 400µl.

   Note: 1-Thioglycerol is viscous. Pipet slowly.
2. Close the tube lid, vortex sample at high speed for 5 seconds, and incubate the sample at 56°C for 30 minutes.
3. Add 200µl of Lysis Buffer to each sample.
4. Close the lid of the tube, and vortex the sample for 5–10 seconds.
5. The sample is now ready for automated DNA extraction using the Maxwell® 16 Instrument. Proceed with cartridge preparation and instrument setup as described in the *DNA IQ™ Casework Pro Kit for Maxwell® 16 Technical Manual* #TM332 (Section 5).

   Note: Store preprocessed sample at room temperature (15–30°C) overnight, if necessary.

5.B. DNA Extraction from Epithelial Fraction Using DNA IQ™ Chemistry and the Maxwell® 16 Instrument

1. To an epithelial fraction of up to 400µl, add 200µl of Lysis Buffer.
2. Close the lid of the tube, and vortex the sample for 5–10 seconds.
3. The sample is now ready for automated DNA extraction using the Maxwell® 16 Instrument. Proceed with cartridge preparation and instrument setup as described in the *DNA IQ™ Casework Pro Kit for Maxwell® 16 Technical Manual* #TM332 (Section 5).

   Note: Store preprocessed sample at room temperature (15–30°C) overnight, if necessary.

6. DNA Extraction from Sperm and Epithelial Fractions Using DNA IQ™ Chemistry on the Maxwell® FSC Instrument

6.A. DNA Extraction from Sperm Fraction Using DNA IQ™ Chemistry and the Maxwell® FSC Instrument

1. To the sperm fraction, add 10µl of Proteinase K solution (18mg/ml), 4µl of 1-Thioglycerol and Casework Extraction Buffer to a total volume of 400µl. Do not exceed a final volume of 400µl.

   Note: 1-Thioglycerol is viscous. Pipet slowly.

2. Close the tube lid, vortex sample at high speed for 5 seconds, and incubate the sample at 56°C for 30 minutes.
3. Add 200µl of Lysis Buffer to each sample.
4. Close the lid of the tube, and vortex the sample for 5–10 seconds.
5. The sample is now ready for automated DNA extraction using the Maxwell® 16 Instrument. Proceed with cartridge preparation and instrument setup as described in the *Maxwell® FSC DNA IQ™ Casework Kit Technical Manual* #TM499 (Section 4).

   Note: Store preprocessed sample at room temperature (15–30°C) overnight, if necessary.

6.B. DNA Extraction from Epithelial Fraction Using DNA IQ™ Chemistry and the Maxwell® FSC Instrument

1. To an epithelial fraction of up to 400µl, add 200µl of Lysis Buffer.
2. Close the lid of the tube, and vortex the sample for 5–10 seconds.
3. The sample is now ready for automated DNA extraction using the Maxwell® FSC Instrument. Proceed with cartridge preparation and instrument setup as described in the *Maxwell® FSC DNA IQ™ Casework Kit Technical Manual* #TM499 (Section 4).

   Note: Store preprocessed sample at room temperature (15–30°C) overnight, if necessary.

7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **genetic@promega.com**

Symptoms	Causes and Comments
Large tight cell pellet (epithelial cells in sperm fraction)	Incomplete lysis of epithelial cells. The proteinase K digestion was incomplete. Use a new vial of proteinase K, and digest the samples for up to 2 hours. Perform the digestion at 56°C.
Large diffuse cell pellet	Large amount of mucus in the sample. Use less sample, or digest the sample with proteinase K for a longer time at 56°C. Diffuse pellets primarily contain cell debris with little DNA.
A bubble of yellow buffer forms within the Separation Solution	Yellow buffer trapped by large amount of cell debris: • Carefully remove any bubbles of yellow solution without disturbing the solid sperm pellet. Take care not to confuse the bubble with the normal yellow color of the sperm pellet. • Perform a longer proteinase K digestion.
Sperm DNA in epithelial fraction or low number of sperm	Degraded sample. Samples containing a large number of lysed sperm due to poor storage conditions will contain some sperm DNA in the epithelial fraction.
	Sperm pellet not covered. Ensure the pellet is fully within the Separation Solution. Add more if necessary.
	Overdigestion of sample. Proteinase K digestion of some samples containing few epithelial cells may result in lysis of some sperm cells. Use less proteinase K, a lower temperature for digestion or a shorter incubation time.
	Too little sample used: • Use more sample. Samples taken more than 72 hours postcoitus have very few sperm. • Consider using the PowerPlex® Y23 System to amplify DNA purified from the sample.

8. References

1. Gill, P., Jeffreys, A.J. and Werrett, D.J. (1985) Forensic application of DNA 'fingerprints'. *Nature* **318**, 577–9.

9. Related Products

Product	Size	Cat.#
DNA IQ™ System	100 reactions	DC6701
	400 reactions	DC6700
Tissue and Hair Extraction Kit (for use with DNA IQ™)	100 reactions	DC6740
Maxwell® FSC Instrument*	1 each	AS4600
Casework Extraction Kit*	100 reactions	DC6745
DNA IQ™ Casework Pro Kit for Maxwell® 16*	48 preps	AS1240
Maxwell® FSC DNA IQ™ Casework Kit*	48 preps	AS1550
Differex™ Separation Solution*	40ml	A8511

*Not for Medical Diagnostic Use.

Accessory Components

Product	Size	Cat.#
DNA IQ™ Resin*	8ml	A8258
	50ml	A8251
Nuclease-Free Water	150ml	P1196
Proteinase K	100mg	V3021
DTT, Molecular Grade (Dry Powder)	5g	V3151
DNA IQ™ Spin Baskets*	50/pack	V1225
ClickFit Microtube, 1.5ml*	100/pack	V4745
Manual Differex™ Magnet	1 each	V1591

*Not for Medical Diagnostic Use.

10. Summary of Changes

The following changes were made to the 6/23 revision of this document:

1. Added text to Section 4.
2. Updated protocol in Section 4.A.

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[a]U.S. Pat. No. 7,320,891.