# LIFluor™ EnhanCE

for use with dsDNA 1000 and dsDNA 20,000 Kits

For the Separation of Double Stranded DNA by
P/ACE™ Fluorescent Capillary Electrophoresis

725824 AB
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# LIFluor EnhanCE

This product is for use with dsDNA 1000 and dsDNA 20,000 kits and a P/ACE MDQ Capillary Electrophoretic System, equipped with a laser-induced fluorescence (LIF) detector.

## Introduction

This package includes one vial of EnhanCE (500 µg in 500 µl of methanol) and the instructions required to use EnhanCE with dsDNA 1000 and dsDNA 20,000 kits.

Upon receipt, store the vial of EnhanCE at -15°C or less.

**NOTE** Before use, let the vial of EnhanCE warm at room temperature for 20 minutes and then mix well.

**IMPORTANT** The EnhanCE is light sensitive. Do not leave it exposed to light when it is not in use.

**IMPORTANT** Always keep the EnhanCE vial capped tightly when not in use. If the vial is left opened, the methanol will evaporate, thus increasing the dye concentration.

## Materials Required but not provided by Beckman Coulter:

- dsDNA 1000 kit (PN 477414)
- dsDNA 20,000 kit (PN 477475)
- LIFluor Performance Test Mix (PN 726022)
- Deionized water
- Parafilm
- Magnetic stir plate and stir bar
- 0.45 µm Syringe Filter with disposable syringe (for aqueous filtering)
- Volumetric Pipet, 20 mL
- Micropipets to deliver 1- 100 µL volume
- Capillary Cartridge, blank (PN 144738)
- 488 nm Notch Filter (PN 144941)
- 520 nm Emission Filter (PN 144940)
- Vials, 2 mL (PN 144980)
- Vial Caps, red (PN 144648)
- PCR Vials (PN 144709)
- PCR Vial Holder (PN 144657)
- PCR Vial Springs (PN 358821)
- PCR Vial Caps, gray (PN 144656)
- DNA Capillary (PN 477477)
- Test Mix λDNA/*Hind III* Fragments (PN 447483)
- dsDNA 20,000 Gel Buffer (PN 477485)
- dsDNA 1000 Gel Buffer (PN 477628)
- dsDNA 1000 Test Mix (PN 477414)

# Using LIFluor EnhanCE with the dsDNA 1000 Kit

## Preparing dsDNA 1000 Gel Buffer with EnhanCE

1. To rehydrate the buffer, add 20 mL deionized water to the Gel Buffer vial.
2. Using a magnetic stir bar and stir plate, stir the solution until the hydrated gel is dissolved.

   It may take up to 24 hours to ensure that the dried gel is completely dissolved.
3. Prior to use, filter the Gel Buffer using a 0.45 µm filter and, if necessary, sonicate it for one minute to remove small bubbles in the Gel.

   Rehydrated Gel Buffer will last for thirty days when stored at 2°C to 8°C.
4. Add 15.0 µL EnhanCE to 20 mL of rehydrated, filtered Gel Buffer and mix well.

**CAUTION** Do not filter the Gel Buffer after the EnhanCE is added.

The EnhanCE is light-sensitive. Do not leave it exposed to light when not in use. It is recommended that aluminum foil be used to cover the Gel Buffer vial to reduce exposure to light. However, EnhanCE may still show deterioration after 10 hours.

**NOTE** The concentration of EnhanCE in the Gel Buffer has been optimized in order to saturate a DNA sample with a concentration of 10 µg/mL. However, for samples with low concentration of DNA (<1 µg/mL), use less EnhanCE (<10 µL) per rehydrated buffer vial for optimal signal.

## Sample Injection

Pressure or electrokinetic injection can be used for dsDNA samples. Pressure injection makes quantitation of DNA possible without sample preparation (i.e. high salt samples). A 10 second pressure injection is recommended. Longer injection times may cause peak shape distortion and migration times to vary. LIFluor Performance Test Mix may be added as an injection marker to DNA Test Mix or your sample. If used, 1 µL of LIFluor Performance Test Mix should be added per 80 µL of sample of Test Mix.

Use Electrokinetic injection with desalted and diluted dsDNA samples. Optimize the injection parameters, depending on the concentration and amount of the sample. As a starting point, use a 2 second injection at 1 KV for a 2.0 µg/mL dsDNA sample.

## Equilibrating a New Capillary

To obtain the best function of the system and capillary, it is recommended to pre-equilibrate and calibrate the capillary by doing the following:

1. Install a DNA capillary into a cartridge so that the total length is 40.2 cm, and the length from the inlet to the window is 30 cm.

**NOTE** Do not expose the capillary ends to air for more than 6 minutes. Excessive exposure to air may permanently damage the inner coating.

2. Calibrate the system as described under "Calibrating the LIF Detector" on this page.
3. Rinse the capillary with prepared Gel Buffer, containing EnhanCE, for 20 minutes at 20 psi.
4. Perform a test separation of the dsDNA test mix per the instructions in "Performing a Test Run using the dsDNA 1000 Test Mix" on page 6.
5. When not in use, rinse the capillary with unused DNA Gel Buffer and store it with both ends submerged in gel at 2-8°C.

## Calibrating the LIF Detector

Whenever the capillary, detector, or laser are changed for the P/ACE MDQ LIF detector, it is recommended to recalibrate the photomultiplier tube.

Calibrate as outlined below:

1. Warm the LIF Performance Test Mix at room temperature for at least 30 minutes
2. Close all open applications except the 32 Karat software.
3. From the Enterprise Screen, open the P/ACE MDQ Instrument for Configuration.
4. Right-click on the LIF Module and select **Configure**.
5. Click **Configure** again.
6. When the **Configured Modules** dialog is displayed (right side), double-click on the LIF Detector icon.
7. On the P/ACE MDQ Instrument Configuration Window, select **LIF Calibration Wizard**.
8. Select **Auto Calibration** and click **Next**.

9. Enter the following calibration parameters:

| | |
|---|---|
| Detector Channel | 1 |
| Target RFU | 62 |
| **Capillary Dimensions:** | |
| Internal Diameter | 100 µm |
| Total Length | 40 cm (dsDNA 1000)<br>50 cm (dsDNA 20,000) |

10. Click **Next**.
11. Place a vial filled with deionized water on position A1 at the Inlet.
12. Place a vial filled with LIF Performance Test Mix on position B1.
13. Place an empty vial on Position A1 outlet.
14. Click **Next**.

The Calibration Wizard will start and a new window will show its progress. When the calibration is complete, a new window will open showing the Calibration Correction Factor (CCF). The CCF value should be between 0.5 and 1.5. A CCF value in excess of 1.5 indicates dirty or misaligned optics. If the CCF value is out of the recommended range, refer to the P/ACE MDQ User's Guide for troubleshooting methods.

## Performing a Test Run using the dsDNA 1000 Test Mix

1. Fill three glass vials with rehydrated, filtered dsDNA 1000 Gel Buffer containing EnhanCE.
2. Sonicate the vials for 5 seconds to remove any bubbles.
3. Place the required vials in the following positions:

| Buffer Inlet Tray | Buffer Outlet Tray |
|---|---|
| A1 - DI $H_20$ | A1 - DI $H_20$ |
| B1 - dsDNA 1000 Gel Buffer | B1 - Waste (Empty) |
| C1 - dsDNA 1000 Gel Buffer | C1 - dsDNA 1000 Gel Buffer |

4. Add 1 mL of deionized water to the dsDNA 1000 Test Mix vial.
5. Vortex the mixture.
6. Transfer 80 µL of the dsDNA 1000 Test Mix mixture to a PCR vial.
7. Aliquot the remainder of the Test Mix in 80 µL volumes and store them at -20°C.
8. Place the dsDNA 1000 Test Mix in the inlet sample tray at position SAI:A1.
9. Use the Methods given in Figures 1 - 3 to analyze the dsDNA 1000 Test Mix.

Initial Conditions | LIF Detector Initial Conditions | Time Program

| | Time (min) | Event | Value | Duration | Inlet vial | Outlet vial | Summary | Comments |
|---|---|---|---|---|---|---|---|---|
| 1 | | Rinse - Pressure | 20.0 psi | 3.00 min | BI:B1 | BO:B1 | forward | filling with gel buffer |
| 2 | | Wait | | 0.00 min | BI:A1 | BO:A1 | | water dip |
| 3 | | Inject - Voltage | 1.0 KV | 2.0 sec | SI:A1 | BO:C1 | Override, reverse polarity | sample injection |
| 4 | | Wait | | 0.00 min | BI:A1 | BO:A1 | | water dip |
| 5 | 0.00 | Separate - Voltage | 7.8 KV | 25.00 min | BI:C1 | BO:C1 | 0.17 Min ramp, reverse polarity | separation |
| 6 | 1.00 | Autozero | | | | | | Autozero |
| 7 | 25.00 | End | | | | | | End |
| 8 | | | | | | | | |

**Figure 1: Time Program in dsDNA 1000 method**

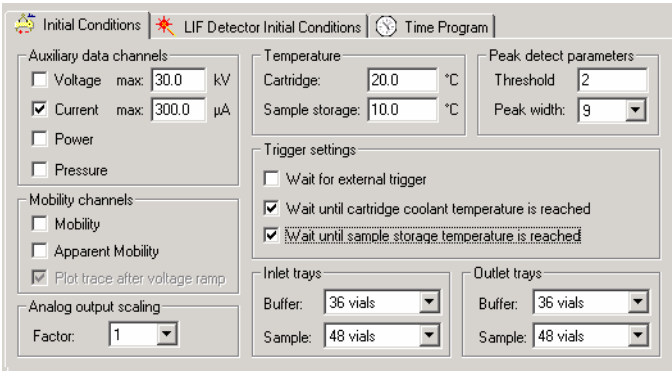


**Figure 2: Initial Conditions in dsDNA 1000 method.**

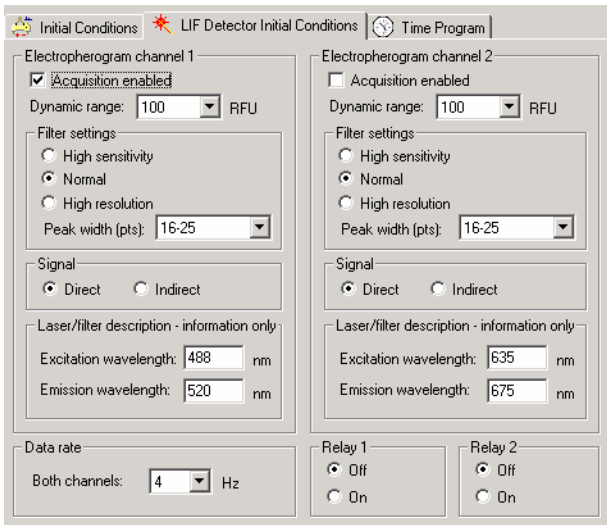


**Figure 3: LIF Detector Setting in dsDNA 1000 method**

**NOTE** During the separation, salts and EnhanCE migrate from one vial of Gel Buffer to the other, changing the composition of the Gel Buffer. It is therefore recommended that the vials of Gel Buffer be replaced with unused Gel Buffer, after 15 injections. If less than 15 µL of EnhanCE is added to the Gel Buffer, change the vials more often.

**NOTE** Due to the viscosity of the Gel Buffer, it is necessary to clean the lever arms, electrodes and the outside of the capillary frequently.

## Checking Your Results

The dsDNA 1000 Test Mix contains Phi-X 174 DNA *Hae III* digest, consisting of 11 DNA fragments. The Test Mix should separate in twenty-five minutes using the recommended method with baseline separation of the 271 and 281 base pairs, as shown in Figure 4.

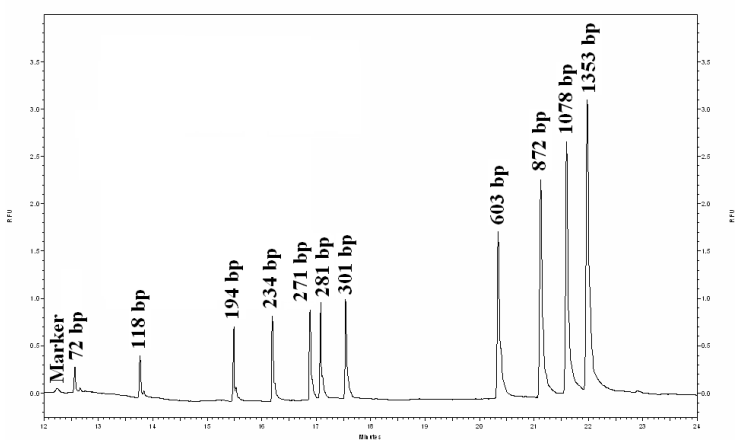


**Figure 4: Electropherogram of dsDNA 1000 Test Mix**

When different dye concentrations are used, variations in migration time will be observed.

If more than 15 µL of EnhanCE is used, the migration time of the dsDNA fragments will increase. But, if less than 15 µL of EnhanCE is used, the dsDNA fragments migrate rapidly. For example, the 72 bp fragment will migrate before the LIF Performance Test Mix, if 2 µL of EnhanCE is used.

If EnhanCE is added directly to the dsDNA sample, it will result in extreme peak tailing. Add EnhanCE to the separation Gel Buffer only.

If any of the dsDNA peaks are followed by a negative baseline upset, then there is not enough EnhanCE in the Gel Buffer to stain all of the dsDNA. Either inject less sample or increase the amount of EnhanCE in the Gel Buffer to eliminate this artifact.

The current should remain fairly stable. Small variations in the current may indicate that the capillary is experiencing temperature fluctuations or the presence of air bubbles in the Gel Buffer. This could result in a noisy baseline, miscellaneous spikes or broad peaks.

# Using LIFluor EnhanCE with the dsDNA 20,000 Kit

## Preparing dsDNA 20,000 Gel Buffer with EnhanCE

1. To rehydrate the buffer, add 18.0 mL deionized water to the Gel Buffer vial.
2. Using a magnetic stir bar and stir plate, stir the solution until the dehydrated gel is dissolved.

   It may take up to 10 hours to ensure that the dried gel is completely dissolved.
3. Prior to use, filter the Gel Buffer using a 0.45 µm filter and sonicate for one minute to remove small bubbles in the Gel.

   Rehydrated Gel Buffer will last for two weeks when stored at 2°C to 8°C.
4. Add 15.0 µL of EnhanCE to the 18.0 mL of rehydrated, filtered Gel Buffer and mix well.

Do not filter the Gel Buffer after the EnhanCE is added. The EnhanCE is light-sensitive.

**WARNING** Do not leave the EnhanCE exposed to light when it is not in use.

It is recommended that aluminum foil be used to cover the vial to reduce exposure to light. However, EnhanCE may still show deterioration after 10 hours.

**NOTE** The concentration of EnhanCE in the Gel Buffer has been optimized in order to saturate a DNA sample with a concentration of 10 µg/mL. However, for sample with low concentration of DNA (<1 µg/mL), use less EnhanCE (<10 µL) per rehydrated buffer vial for an optimal signal.

## Sample Injection

Pressure of electrokinetic injection can be used for DNA samples. Pressure injection makes quantitation of DNA possible without sample preparation (i.e. high salt samples). Five second pressure injection is recommended. Longer injection times may cause peak shape and migration times to vary. LIFluor Performance Test Mix may be added as an injection marker to the DNA Test Mix or your sample. If used, 1 µL of LIFluor Performance Test Mix should be added per 80 µL of sample of Test Mix.

Electrokinetic injection should be used with diluted and desalted dsDNA samples. The injection parameters should be optimized depending on the concentration and amount of dsDNA.

## Performing a Test Run using the dsDNA 20,000 Test Mix

1. Install a DNA capillary into a cartridge so that the total length is 50.2 cm, and the length from the inlet to the window is 40 cm.

**NOTE** Do not expose the capillary ends to air for more than 6 minutes. Excessive exposure to air may permanently damage the inner coating.

2. Before performing a test run as outlined on the following pages, perform a system calibration. See "Calibrating the LIF Detector" on page 4.
3. Equilibrate the capillary by rinsing it for 20 minutes at 20 psi, with dsDNA 20,000 Gel Buffer containing EnhanCE.
4. Fill three 2 mL vials with rehydrated, filtered dsDNA 20,000 Gel Buffer, containing EnhanCE.
5. Sonicate the vials for 5 seconds to remove any bubbles.
6. Place the required vials in the following positions:

| Buffer Inlet Tray | Buffer Outlet Tray |
|---|---|
| A1 - DI $H_20$ | A1 - DI $H_20$ |
| B1 - dsDNA 20,000 Gel Buffer | B1 - Waste (Empty) |
| C1 - dsDNA 20,000 Gel Buffer | C1 - dsDNA 20,000 Gel Buffer |

7. Transfer 50 µL of the dsDNA 20,000 Test Mix into a PCR sample vial, and cover the top with parafilm.
8. Heat the sample at 65° for 5 to 7 minutes in a water bath.
9. Cool this sample down immediately in an ice-water bath for 3 to 5 minutes.
10. Use the methods in Figures 5 - 7, to analyze the dsDNA 20,000 Test Mix.

**NOTE** During the separation, salts and EnhanCE migrate from one vial of Gel Buffer to the other, changing the composition of the Gel Buffer. It is therefore recommended that the vials of Gel Buffer be replaced with unused Gel Buffer, after 15 injections. Change the Gel Buffer more often, if less than 15 µL of EnhanCE is added to the Gel Buffer.

**NOTE** Due to the viscosity of the Gel Buffer, it is necessary to clean the lever arms, electrodes and the outside of the capillary frequently.

Initial Conditions | LIF Detector Initial Conditions | Time Program

| | Time (min) | Event | Value | Duration | Inlet vial | Outlet vial | Summary | Comments |
|---|---|---|---|---|---|---|---|---|
| 1 | | Rinse - Pressure | 20.0 psi | 3.00 min | BI:B1 | BO:B1 | forward | filling with dsDNA gel against vial filled with water |
| 2 | | Wait | | 0.00 min | BI:A1 | BO:A1 | | cleaning capillary tips in vials filled with water |
| 3 | | Inject - Pressure | 0.5 psi | 10.0 sec | SI:A1 | BO:B1 | Override, forward | dsDNA injection against vial filled with gel |
| 4 | | Wait | | 0.00 min | BI:A1 | BO:A1 | | cleaning capillary tips in vials filled with water |
| 5 | 0.00 | Separate - Voltage | 10.0 KV | 25.00 min | BI:C1 | BO:C1 | 0.17 Min ramp, reverse polarity | separation between gel filled vials |
| 6 | 1.00 | Autozero | | | | | | |
| 7 | 25.00 | End | | | | | | |
| 8 | | | | | | | | |

**Figure 5: Time Program in dsDNA 20,000 method**

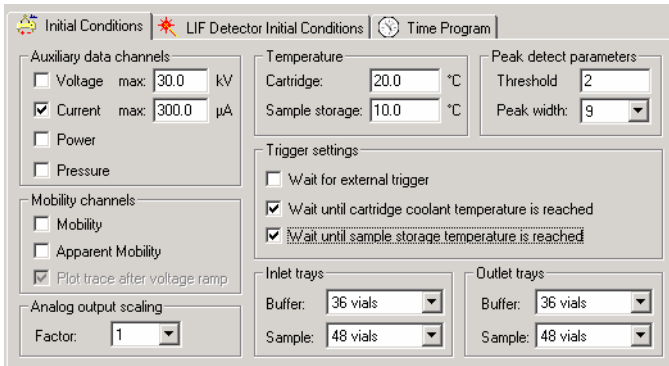


**Figure 6: Initial Conditions in dsDNA 20,000 method**

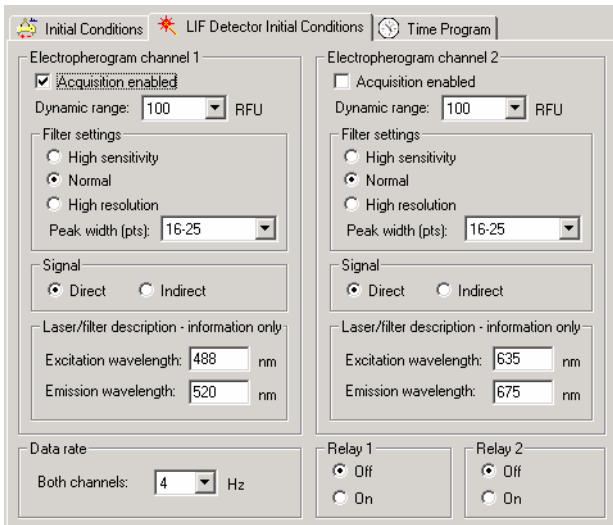


**Figure 7: LIF Detector Setting in dsDNA 20,000 method**

## Checking Your Results

The dsDNA 20,000 Test Mix contains λ *Hind III* digest, consisting of 8 DNA fragments. The Test Mix should separate in twenty-five minutes using the recommended method as shown in Figure 8. The 125 base pair fragment may be difficult to detect, due to its small size.

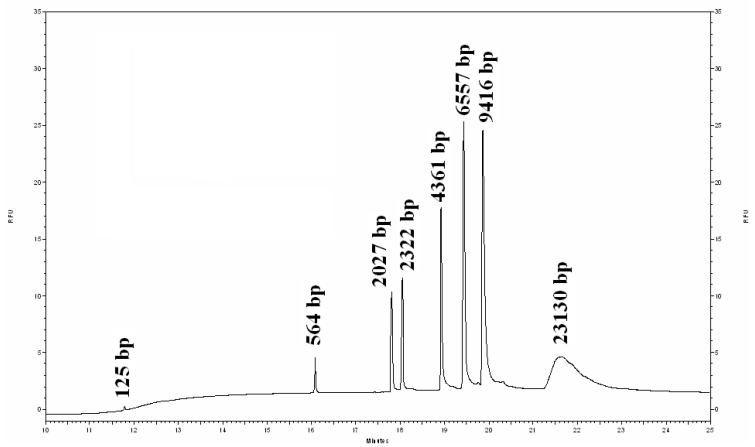


**Figure 8: Electropherogram of dsDNA 20,000 Test Mix**

Variations in migration time can be observed when different dye concentrations are used.

If more than 15 µL of EnhanCE is used, the migration time of the dsDNA fragments will increase. But, if less than 15 µL of EnhanCE is used, the migration time of the dsDNA fragments will decrease.

If EnhanCE is added directly to the dsDNA sample, it will result in extreme peak tailing. Add EnhanCE to the separation Gel Buffer only.

If any of the dsDNA peaks are followed by a negative baseline upset, then there is not enough EnhanCE in the Gel Buffer to stain all of the dsDNA. Either inject less sample or increase the amount of EnhanCE in the Gel Buffer to eliminate this artifact.

The current should remain fairly stable. Small variations in the current may indicate that the capillary is experiencing temperature fluctuations or the presence of air bubbles in the Gel Buffer. This could result in a noisy baseline, miscellaneous spikes or broad peaks.

## Troubleshooting Guide

| Problem | Possible Cause | Corrective Action |
|---|---|---|
| Low Current | Plugged Capillary | Replace the Gel Buffer in the capillary by rinsing it with deionized water at 70 psi for 1 minute. |
| Broad peaks or changing migration times | Dried gel on electrodes | Clean electrodes, ends of capillary and P/ACE lever arms. |
| | Deteriorating Test Mix or Gel Buffer | Replace the Test Mix or Gel Buffer as needed. |
| No peaks | Plugged or dried capillary | Replace Gel Buffer inside the capillary as described above or replace the capillary. |
| | Broken capillary | Replace the capillary. |
| Spikes in the electropherogram | Bubble in the Gel Buffer | Make sure that the reconstituted buffer is at room temperature and that air is removed, by degassing or sonicating the gel buffer vials before use. |
| Noisy baseline | Microparticles in Gel Buffer | Prepare fresh filtered Gel Buffer. |
| Decrease in peak height | Dye deterioration | Prepare fresh EnhanCE-gel solution. Protect gel/dye solution from light. |
| Migration time change | Dye deterioration | Prepare fresh EnhanCE-gel solution. Protect EnhanCE-gel solution from light. |
| | Non-homogeneous dye/gel solution | Make sure that the EnhanCE-gel solution has been mixed well. |
| | Dye/gel evaporation | Prepare fresh EnhanCE-gel solution. |
| Current change | Dye deterioration | Prepare fresh EnhanCE-gel solution. Protect EnhanCE-gel from light. |
| | Dirty electrodes | Clean electrodes and P/ACE lever arms. |
| Low peak signal | Sample concentration was too low | Increase injection amount. |
| | Dye concentration was too high | Reduce dye volume added to gel for diluted sample. |
| | Capillary window not aligned with detector | Check the cartridge to make sure that the window is aligned correctly. |
| High peak signal with poor peak resolution | Sample overload | Dilute the sample and/or inject less sample. Reduced sample loads will improve both resolution and peak efficiency. |