

H/D-X PAL™

Hydrogen-Deuterium Exchange PAL



Technical Note # 1001

An Advanced Automation, Scheduling & Injection Platform for H/D-X Sample Preparation

The H/D-X PAL™ Platform is an automated scheduling and experimental execution system providing the user, ease of use, high reproducibility and exceptional data quality.

H/D-X PAL™ is an easy-to-use system that provides an automated process for the scheduling and experimental execution of H/D-X experimental workflow. By use of the advanced LEAP Shell scheduling software; experimental design is simplified and reliable. Synchronized reagent addition and sample labeling steps are automatically scheduled to increase throughput and produce the highest quality data.

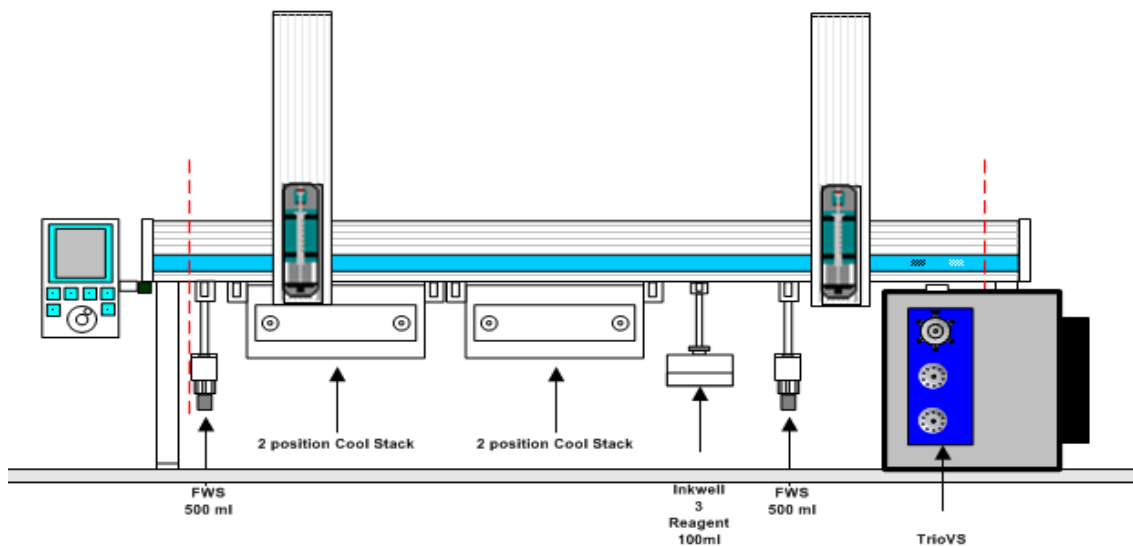


Challenges of Hydrogen-Deuterium Exchange –Sample Prep

Hydrogen Deuterium Exchange is an experimental technique to obtain structural data on proteins. The technique relies on the accurate measurement of the degree of labeling of a protein by deuterated hydrogen during a precisely measured labeling interval. The labeling reaction is stopped by addition of a quenching reagent. Loss of the label between the quench step and analysis (*back-exchange*) is minimized by keeping the reactants at a temperature close to 0°C.

- The technique is difficult to perform manually due to the demands for precise timing and reproducing exactly the same experimental conditions. Automation addresses both of these challenges.
- LEAP has developed a robotic solution for the sample preparation and introduction to the LC-MS.
- There are multiple temperature controlled zones on the workstation: Cooled stack(s) for the samples and labeled reactants, and a Cool Chromatography Module which contains the injection valve, the column selection valves and the columns themselves. These zones are independently programmed to temperatures in the range of 1°C to 40°C.
- The H/D-X PAL™ seamlessly combines versatile hardware and powerful software to automate the difficult timing and reaction mechanisms for superior results.

High Resolution, Precise, Robotic Liquid Handling



System Design Drawing: 120 mm X-Y PAL Rail with Sample Cooling Reagent Wells, Syringe, Wash Station, Temperature Controlled- Cool Chromatography Module and Dual Head Dispense Capability

The Reaction Process:

The H/D-X technique relies on accurate timing of the labeling step, and in maintaining low temperatures for all steps after the labeling reaction has been quenched.

Most experiments require labeling times in a range from 10 seconds to 8 hours. Precision in the timing is critical, especially for the shorter reaction times. The H/D-X PAL™ allows reaction scheduling interruption and in-process reactant injection to the chromatography system. By use of intelligent logic timing conflicts are avoided.

One of the biggest challenges to manually performing this technique is the correct timing of each step. LEAP has developed a scheduling application using the proprietary software LEAP Shell™, which will automate the labeling of samples, as well as injection to the LC-MS analysis system.

A typical experiment would consist of a dozen time points for each protein sample.

The H/D-X PAL™ system will schedule the experiments and perform each of the steps for each time point and sample:

Labeling

The protein is mixed with a Deuterium reagent and incubated for a precisely timed interval at ambient temperature.

Quenching

The labeling reaction is stopped by transferring a volume of the mixture to an excess of quenching reagent in a pre-cooled vessel (0°C).

Injection

A measured portion of the quenched reactants is injected into a multi-column, multi-valve LC-MS system. This step is synchronized with the analytical system using hardware signals. The PAL will inject samples as the LC-MS system becomes ready. Labeled and quenched reactants are held in the cooled stack until they have been injected and analyzed.

H/D-X PAL Automation Software Powered by LEAP Shell

**A Solution for the Challenges to Produce
the Highest Quality Data Sets:**

- **Precise data point timing**
- **Reproducible experimental conditions**
- **Precise temperature control**
- **Programmable temperature zones**
- **Complex valve control of LC injection**
- **Elimination of timing conflicts**



Key Features of H/D Exchange PAL Scheduler Application –

Automation Driven Application Software:

- Easy Entry of the labeling experiments in ascending order of labeling time.
- No limit to the number of samples which can be handled in a single experiment. Label experiments are run concurrently to optimize throughput. Replicates are also supported.
- Run-time display of the “Status” for each sample as it progresses through the experiment, e.g. “Labeling”, “Quenched”, “Injected”.
- The speed of the PAL is monitored by the program and timing adjustments are made on the fly to maintain incubation accuracy. (e.g. No need to worry about timing if PAL wash parameters are changed)
- Log file output of all experimental conditions, actual labeling and injection times for each sample.
- Long labeling time experiments are automatically set up first to get the process started as soon as possible.
- Samples are injected as the LC-MS system comes ready.
- No need for the operator to worry about timing conflicts for the labeling steps. These are resolved by the program on the fly.

The LEAP Shell Automation Architecture

The LEAP Shell driven H/D-X Scheduler program can save and recall an unlimited number of protocols for easy execution of routine experiments. Dozens of samples may be scheduled for increased throughput and previously unattainable data quality.

Intuitive User Interface

- Microsoft Windows™ Operator Interface
- Single View User Interface
- Selection of the Desired Experimental Parameter
- Simply Integrate Sample Preparation with Injection and Mass Spectrometry Analysis
- Save and Load Routine Sample Preparation Programs

HD-x Twin

Profile Tools Log Out Help

Save Save as... Add samples... Fill Columns... Select vial...

Sample Lists:

- test
- testing
- 3 minutes 7 samples
- 3 minutes 4 samples

SampleID	Shell Method	Well	hr	min	sec
1 LSSample001	HDx Prep 3 min	1	0	0	10
2 LSSample002	HDx Prep 3 min	1	0	0	30
3 LSSample003	HDx Prep 3 min	1	0	1	4
4 LSSample004	HDx Prep 3 min	1	0	2	40
5 LSSample005	HDx Prep 3 min	1	0	4	40
6 LSSample006	HDx Prep 3 min	1	1	0	12
7 LSSample007	HDx Prep 3 min	1	2	30	40
8 LSSample008	HDx Prep 3 min	1	2	30	40
9 LSSample009	HDx Prep 3 min	1	2	30	40
10 LSSample010	HDx Prep 3 min	1	2	30	40
11 LSSample011	HDx Prep 3 min	1	2	30	40
12 LSSample012	HDx Prep 3 min	1	2	30	40
13 LSSample013	HDx Prep 3 min	1	2	30	40
14 LSSample014	HDx Prep 3 min	1	2	30	40
15 LSSample015	HDx Prep 3 min	1	2	30	40
16 LSSample016	HDx Prep 3 min	1	2	30	40
17 LSSample017	HDx Prep 3 min	1	2	30	40
18 LSSample018	HDx Prep 3 min	1	2	30	40
19 LSSample019	HDx Prep 3 min	1	2	30	40
20 LSSample020	HDx Prep 3 min	1	2	30	40

Sample Scheduling Selection View

H/D-X PAL™ Specifications

- Incubation Times from Several Hours Down to 5 Seconds Minimum - User Selectable
- Runtime Display accuracy to one (1) second
- Temperature Control +/- 1.0°C
- Temperature Range 1.0°C to 40.0°C
- Dispense Volume Range Typically 1-100 ul, User Application Selectable to 5000ul
- Electrical 120 VAC, 60 Hz or 220 VAC, 50Hz
- Dimensions/Weight 120cm (w) x 38.5cm (d) x 64.8cm (h) ~ 10kg



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Method Parameters

Protein Sample Plate	CStk1-02
Labeling Plate	MT1-Fmt
Quench Plate	CStk1-01
First Reaction Vial	1
Protein Volume	2
D2O Volume	30
Quench Volume	32
Transfer Volume	32
Inject Volume	60
Digestion Time (sec)	20
Filling Speed for Inj PAL	50
Filling Speed for Prep PAL	2
Dispensing Speed for Inj PAL	80
Dispensing Speed for Prep PAL	10
Mixing Strokes	1
Syringe Cleaning Strokes Solvent 1	1
Syringe Cleaning Strokes Solvent 2	0
Valve washes Solvent 1	1
Valve washes Solvent 2	0
Injector	LC Vlv1
Labeling Record Filename	7-8-08_1.txt
LC Runtime (min)	2
Injection Sequence	Keep Sample List sequence

Experimental Parameters Selection View

Additional Information:

H/D-X PAL™ is available worldwide from LEAP Technologies, Inc.

To request further information please contact
LEAP Technologies at: +1-919-929-8814
PO Box 969
Carrboro, NC 27510 USA www.leaptec.com

LEAP Technologies also offers many other solutions for sample prep automation including:

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