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INSTRUCTION MANUAL OF LDH AGE KIT

(KT-LDA01/ KT-LDA05/ KT-LDA10)

Manufactured and Marketed by:

TechResource

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Kit components

Sr. No.	Components	Store at
1.	30 mL 1% Buffered agarose	4°C
2.	30 mL 5 X Tank buffer	4°C
3.	200 µL Loading dye buffer	4°C
4.	20 mL Substrate buffer	4°C
5.	3.5 mL NAD (oxidiser) 10 mg/mL	-20°C
6.	1.2 mL PMS (stabilizer) 1 mg/mL	-20°C
7.	10 mL NBT (electron acceptor) 1 mg/mL	-20°C
8.	400 mL D/W	4°C

Equipments and consumables required but not provided in the kit:

- Horizontal Electrophoresis unit,
(maximum size 7 x 8.5)
- Power supply
- Freshly minced chicken (brain/liver/ heart/ kidney)
- Tissue homogenizer
- Clean scissors & forceps
- Centrifuge (for microfuge tube)
- Glass pipettes (1, 5, 10 ml) 1 each
- One 25 ml measuring cylinder
- Micropipettes-
0.5-10 µL
5-50 µL
10-100 µL
100-1000 µL
- Tips
- Refrigerator
- 37 °C incubator

Preparation instructions:

1X tank buffer:

0.5X tank buffer is to be prepared from 5X tank buffer by diluting with D/W.

Table :

	1 Expt.	5 Expts.	10 Expts.
D/W	270 mL	1350 mL	2700 mL
5X Tank buffer	30 mL	150 mL	300 mL
Total	3000 mL	1500 mL	3000 mL

Store 0.5X tank buffer at 4°C.

Activity staining solution:

Mix the following and store in a dark bottle at -20°C
17 mL Substrate buffer
3 mL NAD oxidizer
0.8 mL PMS stabilizer
8 mL NBT

Choice between TAE and TBE

The choice between TAE and TBE can be made based on the following properties

- TAE has low buffering capacity and will get exhausted if electrophoresis is carried out for prolonged times.
- TBE is more expensive than TAE.
- Double stranded DNA fragments migrate faster through TAE than through TBE.
- The resolving power of TAE is better for higher molecular weight and for supercoiled DNA. TBE is better for lower-molecular weight DNA.

Choice of Agarose concentrations

Agarose conc in gel (% w/v)	Range of separation of linear DNA	V grad-ient (V/cm)	Notes
0.3	5-60	5	Very fragile. Opt for PAGE if possible
0.6	1-20	5	
0.7	0.8-10	5	
0.8	0.5-7	10	BPB runs at approx. 1 kb, XC at approx. 4 kb
0.9	0.5-7	10	
1.0	0.5-6	10	
1.2	0.4-6	10	BPB runs at approx. 0.5 kb
1.5	0.2-3	10	
2.0	0.1-2	20	
3.0	0.1-1	20	Pour agarose at 55°C in pre warmed tray

Troubleshooting Guide

Indication	Cause	Remedy
1. Problems with power supply		
Power supply will not switch 'ON'. i.e. light on mains switch/LED indicator does not come 'ON'.	Check 230 V mains supply	Restore the mains supply
	Power supply fuse blown	Try replacing with correct fuse only once
	Fuse blows again	Contact the manufacturer of the power supply
2. Problems with horizontal electrophoresis unit		
Power supply 'ON' but no movement of samples/ bubbles in the buffer	Cathode and anode are not connected to the right output terminals.	Connect the cathode and anode to the right output terminals i.e. red to red and black to black
	Electrical connecting cords are not properly inserted.	Insert the electrical connecting cords properly.
	The connecting cords are damaged.	Use new connecting cords
	Low level of buffer in the apparatus	Raise the level of buffer in the apparatus such that the circuit is formed.
	Presence of cello tape in tray	Remove cello tape and check
	Oxidation/deposit formation on the contact terminals/ connectors	Disconnect the instrument. Clean the terminals. Reconnect the instrument and try again.
	Protein was electrophoresed off the gel	Electrophorese the gel for less time, at lower voltage
No bands seen	To low concentration of sample	Concentrate the sample before loading
	sample was degraded	Avoid contamination
	Activity Stain not working	Make sure the Activity stain was prepared correctly and stored in a freezer at -20 °C

Protocol summary:

- Sample preparation is to be done by mincing freshly dissected material in D/W. (1 g tissue: 2 mL D/W)
- Transfer 1 mL of the slurry to microcentrifuge tubes and incubate at RT for 30 mins.
- Centrifuge 10 mins at RT at 2000 rpm.
- Collect supernatant and recentrifuge
- Clean and dry the electrophoresis apparatus.
- Prepare 0.5X tank buffer and chill in refrigerator.
- Seal the sides of the tray with tape.
- Place the comb in tray.
- Melt agarose in hot water bath.
- Cool to approximately 50°C.
- Pour the gel and allow to set for 15-20 mins.
- Remove the tape and place the tray in apparatus.
- Pour chilled 0.5X tank buffer into the apparatus such that buffer should be 2-4 mm above the gel.
- Remove the comb gently.
- Before loading the samples, add loading buffer to it (1:1).
- Load the sample and connect the apparatus to power supply.
- Start the power supply. Adjust the voltage and current as per your requirement.
- Switch off the power supply when the blue tracking dye reaches towards the end of the gel.
- Load the samples. Connect to a power supply and carry out electrophoresis at required Voltage.
- Switch the power supply off when the blue tracking dye reaches the bottom of the gel.
- Prepare activity staining solution.
- Remove the gel from the assembly and stain the gel with activity staining solution.



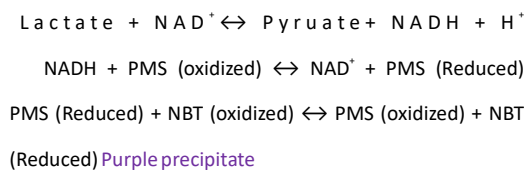
23) Interpretation of zymogram.



LDH zymography

Introduction:

Lactate dehydrogenase (LDH) exists in five forms (i.e. Isozymes). Each one has a slightly different structure that makes their separation feasible. Zymography is a poly-acrylamide gel electrophoretic technique, for qualitative detection of enzyme activity. LDH isozymes are separated on a PAGE and activity staining is performed on the gel to visualize separated isozymes. In LDH activity staining, the enzymes present on in the gel react with substrate and a series of reactions occur to form a purple coloured precipitate. The reactions occur as follows:



[PMS: PhenazineMethosulfate,
NBT:Tetranitrobluetetrazolium]

Theory:

Electrophoresis is a technique in which charged molecules migrate from cathode to anode under influence of electric current. Agarose is a polysaccharide obtained from red algae. Agarose forms inert matrix through which charged molecules migrate towards anode. Smaller molecules move faster compared to larger ones, in a process called sieving. Higher the percentage of agarose lower is the pore size and vice versa. Most agarose gels are made between 0.7% and 2% of agarose depending on size of DNA/RNA to be separated. The DNA/RNA can be visualized by adding ethidium bromide (EtBr) in the gel or loading buffer.

Various factors can affect the migration of nucleic acids during electrophoresis: the percentage of agarose, ionic

strength of the buffer, the voltage applied, the concentration of EtBr and size of nucleic acid being electrophoresed.

Detailed Protocol:

Assembling and sealing the apparatus:

- 1) Rinse all items, beakers, measuring cylinders, pipettes and electrophoretic apparatus with distilled water, dry and use.
- 2) Prepare 0.5 X tank buffer as mentioned in table 1. Cover with a plastic sheet and chill in the refrigerator.

Sealing the tray:

- 3) Tape both the ends of the tray making sure that the tape is high enough and place it on a levelled surface.

Casting the gel:

- 4) If using the comb stand: place the comb stand on the tray.
If using the fixed height comb: insert the comb in the slots provided.
- 5) Melt the agarose in a hot water bath.
- 6) Cool the agarose to approximately 50°C and add EtBr (final concentration 0.5 µg/ml) if desired. Pour the warm solution into the tray to a gel thickness of about 4-5 mm. Allow the gel to set for minimum 15 mins.
- 7) Carefully remove the tape and place the gel tray in the apparatus such that the wells are closer to the cathode (black connector).
- 8) Pour 0.5X tank buffer into the apparatus such that buffer should be 2-4 mm above the gel.
- 9) Remove the comb gently.
- 10) Mince the freshly removed chicken brain, kidney, liver &

Sample preparation:

heart in a tissue homogenizer using distilled water.(For every gram of chicken tissue use 2 mL of distilled water).Make a fine slurry.

- 11) Transfer 1 mL of the slurry to 1.5 mL microfuge tubes and keep it out at room temp. (RT) for 30 min to allow enzyme to diffuse out of the cells.
- 12) Centrifuge for 10 mins. at 2,000 rpm. Collect the supernatant in fresh 1.5 mL tubes and re-centrifuge as above. Collect the supernatant and store the extract at RT

Running the gel:

- 13) Load 20 µL of the sample per well.
 - 14) Connect the apparatus to power supply. Start the power supply. Adjust the voltage and current as per your requirement. (For a small apparatus of 3 x 10 cm adjust to a constant voltage of 50-70) *Check if the samples are migrating in the correct direction.*
 - 15) Switch the power supply off when the blue tracking dye reaches towards the end of the gel.
 - 16) Remove the gel tray from the apparatus, drain out all the buffer carefully and remove the gel from the tray and place in a staining dish.
- ### Activity staining:
1. Prepare the working staining solution in a tray, by mixing substrate buffer, electron acceptor, stabilizer and oxidizer (as mentioned in the preparation).
 2. Carefully slide the gel into the tray containing activity staining solution. Incubate for 10-15 mins. in the dark at 37C or until bands develop. Observe the zymogram for blue bands against white background.
 3. Discard the stain and observe the zymogram on a white light illuminator.

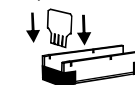
PS*- Power supply

Flow chart

1) Sealing of the tray



2) Place comb



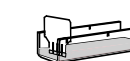
3) Melt agarose



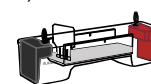
4) Pour agarose into the tray



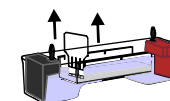
5) Remove the cello tape



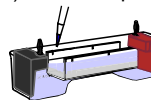
6) Place in tank



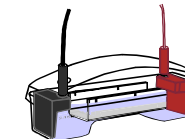
7) Pour buffer & remove comb



8) Load samples



10) Cover with lid & connect to PS*



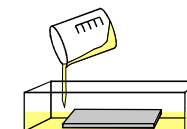
11) Stop PS. Remove gel and place in tray



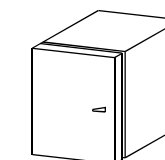
12) Make activity staining solution



13) Pour the activity staining solution in the tray



14) Keep in the dark at 37°C



14) Observe the gel after 10-15 mins

