MDH Storage/Stability Protocol:

Instructions – Storage of recombinant MDH protein

MDH, like most enzymes/proteins is subject to aggregation, denaturation and precipitation. To better support the MCC project we have conducted a series of studies to optimize storage conditions described in detail below.

Never freeze solutions of MDH without additives. MDH will aggregate and precipitate out of solution after even one freeze/thaw cycle in a simple buffer (like phosphate); wgMDH or hMDH1 will lose 40-65% of activity.

Stability at 4°C (refrigerator)

Purified proteins eluting from Ni-NTA columns should be dialyzed against 10 mM potassium phosphate, 150 mM NaCl, 0.1 mM EDTA, pH 8.0, to remove the imidazole used to elute the protein. Imidazole can, in some cases cause protein to precipitate. Therefore it is a good idea to remove imidazole by dialysis routinely, irrespective of your downstream application.

Protein concentration and dialysis: While it might seem that more is better, higher protein concentrations (1.0 - 2.0 and greater) may often result in aggregation and protein loss. Be aware of this as you work with your proteins. Some of the MCC MDH clones will easily express and purify at much higher concentrations. Even left overnight at this concentration, significant precipitation may occur overnight.

High protein concentrations (1.5 mg/mL and greater) may also result in aggregation and precipitation. wgMDH, hMDH1, hMDH2, and pFalci MDH are all high to moderate high protein expressors routinely producing 20-100 mg of protein from a 1 L culture. Left overnight at concentrations >1.2-2.0 mg/mL, significant precipitation may occur. A best practice is to test the limits of concentration of the isozyme/mutants you are working with. Without that knowledge, the rule of thumb is to dilute to 0.9-1.0 mg/mL immediately after purification with a dialysis buffer (see below). After dilution, dialyze the protein with 1-2 changes of that dialysis buffer (20-50 volume of dialysis buffer to protein). To remove imidazole for rebinding to Ni-beads or other sensitive work (i.e., fluorescence), dialyze the protein with 3-4 changes of dialysis buffer (50-100 volumes of dialysis buffer to protein).

Storage at 4ºC: wgMDH is quite stable. Over short term (2-4 weeks) a dialyzed wgMDH sample will stay in solution and with only a slight loss of activity. Longer storage at 4ºC in KPi buffer (see table below) will maintain most of the activity (only 5-8% loss). However, not all MDH isoforms are as stable. A solution of MDH1 will become slightly turbid losing ~30% or more of its activity stored in simple KPi buffer over 2-4 weeks and nearly 80% of activity is lost at 4ºC in 90 days. However, there are three solutions that without freezing maintain MDH1 activity:

- 25% glycerol in KPi buffer will retain 80% of its activity over 90 days.
- 10% glycerol, 50 mM NaCl, 1 mM β-mercaptoethanol in 10 mM K phosphate, pH 8.0 will retain 90-80% of activity over 90 days.
- 5% glycerol, 95 mM ammonium sulfate, 100 mM NaCl 0.125 mM β-mercaptoethanol, 10 mM K phosphate, pH 8 retains nearly all activity but has a much higher ionic composition and may interfere with downstream use.

Storage at -20ºC or -80ºC: Freezing MDH is possible in the right solution. While wgMDH is most stable, both wgMDH and MDH1 were most stable in 10 mM phosphate buffer containing 0.1 mM EDTA and either 10 or 25% glycerol. Another effective buffer is 10% glycerol, 50 mM NaCl, 1 mM □-mercaptoethanol (GNM) in 10 mM K Phosphate, pH 8.). With any of these three buffers less than 5% of activity was lost over 90 days while at −80ºC. MDH1 is more stable in the more complex GNM buffer by a small amount. Freezing at −20°C will result in 10-20% more loss of activity in the same buffers than when storing at −80°C.

Both wgMDH and MDH1 are reasonably stable in:

- 10 mM KPi buffer containing 0.1 mM EDTA and 10-25% glycerol (less than 5% of activity was lost over 90 days while at -80ºC
- GNM buffer (10% glycerol, 50 mM NaCl, 1 mM β-mercaptoethanol, 10 mM K Pi, pH 8.0). Less than 5% of activity was lost over 90 days while at -80ºC. MDH1 is slightly more stable in GNM buffer.

First rule: Never freeze your MDH solution without additives. MDH will precipitate after even one freeze thaw in a simple buffer (like KPi buffer) or even worse with imidazole. One cycle of freeze that of either watermelon glyoxal MDH (wgMDH) or human cyto MDH (MDH1) will lose 40-65% of the activity when frozen at 4°C. The same loss of activity will happen if frozen at -70° to -80°C.

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Storage Recommendations:

MDH Isozyme	Storage Duration	Temp	Storage Solution
wgMDH			
	1-4 weeks	4ºC	KPi buffer: 10 mM KPi,150 mM NaCl, 0.1 mM EDTA, pH 8.0
	3 weeks – 3 months	-80ºC	10 mM K-phosphate, 0.1 mM EDTA, 20% glycerol, pH 8.0
All other MDH			
	1-4 weeks	4ºC	10 mM K-phosphate, 0.1 mM EDTA, 20% glycerol, pH 8.0
	2 weeks – 3 months	-80ºC	10% Glycerol, 50 mM NaCl, 1 mM □-ME in 10 mM K Phosphate, pH 8.0

- * Dialyzing against the storage solution may not be practicable as dialyzing against 5-10 dialysate volume with 3 or more changes may make using these buffers problematic. Another answer is to dialyze against a 10 mM KPi buffer with 0.1 mM EDTA and adding 2 or 4X of the remaining components made in the same buffer (10 mM KPi buffer with 0.1 mM EDTA).