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NAD+/NADH检测试剂盒(WST-8法)

产品编号	产品名称	包装
S0175	NAD+/NADH检测试剂盒(WST-8法)	100次

产品简介:

- 碧云天的NAD+/NADH检测试剂盒(WST-8法) (NAD+/NADH Assay Kit with WST-8)是一种基于WST-8的显色反应，通过比色法来检测细胞、组织或其它样品中NAD+ (氧化型辅酶I)和NADH (还原型辅酶I)各自的量、比值和总量的检测试剂盒。
- NAD+/NADH以及NADP+/NADPH的传统检测方法是检测NADH或者NADPH在340nm处吸收波长的变化，该方法灵敏度较低并易受样品中有类似紫外吸收物质的干扰，并且在紫外检测过程中通常需要加大检测样品量以弥补NADH在340nm处吸光度过小的不足，因此该传统检测方法具有很大的局限性。
- WST-8是MTT的一种升级替代产品，和MTT或其它MTT类似产品如XTT、MTS等相比有明显的优点。首先，MTT被一些脱氢酶还原生成的formazan不是水溶性的，需要有特定的溶液来溶解；而WST-8和XTT、MTS产生的formazan都是水溶性的，可以省去后续的溶解步骤。其次，WST-8产生的formazan比XTT和MTS产生的formazan更易溶解。再次，WST-8比XTT和MTS更加稳定，使实验结果更加稳定。另外，WST-8和MTT、XTT等相比，线性范围更宽，灵敏度更高。
- WST-8 和 WST-1 相比，检测灵敏度更高，更易溶解，并且更加稳定。
- 本试剂盒使用便捷，无需分离纯化细胞、组织或其它样品中的NAD+和NADH，并且能特异性检测NAD+和NADH，而不检测NADP+和NADPH。本试剂盒可以检测含量低至0.25μM (5pmol)的NAD+或NADH，在0.25μM (5pmol)至10μM (200pmol)之间呈现良好的线性关系。
- NAD (Nicotinamide adenine dinucleotide，烟酰胺腺嘌呤二核苷酸)是所有细胞中都存在的一种辅酶，包括NAD+ (氧化型)和NADH (还原型)两种形式。NAD+既是氧化还原反应过程中传递电子的辅酶，又可以作为很多酶的底物来参与细胞内反应。例如Sirtuins家族的Sirt1等去乙酰化酶就需要以NAD+作为底物进行去乙酰化反应来调控蛋白的乙酰化水平从而参与细胞的生命活动过程。NAD+在细胞和体内发挥着重要的功能，其合成和降解及其产物参与细胞凋亡、代谢调控和基因表达的调控等，并且NAD+的减少是细胞死亡的主要因素之一。虽然NMNAT (nicotinamide mononucleotide adenylyltransferase)是NAD+的合成酶，包括Nmnat1、Nmnat2和Nmnat3，但NAMPT (Nicotinamide phosphoribosyltransferase)通常被认为是NAD+合成的限速酶。NAD+在调节细胞氧化还原状态方面的重要性以及调控信号通路及转录方面的功能，使得NAD+及其合成和消耗的酶成为多种疾病的潜在药物靶点。
- 本试剂盒可检测样品中的NAD+、NADH以及它们的比值，具体原理如下:
  a. 测定NAD+和NADH的总量：乙醇(Ethanol)在乙醇脱氢酶(Alcohol dehydrogenase，ADH)的作用下氧化生成乙醛(Acetaldehyde)，在这一反应过程中NAD+被还原为NADH；生成的NADH在电子耦合试剂1-mPMS (1-Methoxy-5-methylphenazinium Methyl Sulfate)的作用下将WST-8还原生成橙黄色的formazan，在450nm左右有最大吸收峰。反应体系中生成的formazan与样品中NAD+和NADH的总量呈比例关系。WST-8法检测NAD+和NADH总量的原理参考图1。
  b. 单独测定NADH的量：60°C水浴加热30分钟后，样品中NAD+会分解而只保留NADH。NADH将WST-8还原成formazan，通过比色法确定反应生成的formazan的量，最终可以确定样品中NADH的量。
  c. 测定NAD+以及NAD+/NADH比值：根据前两步检测获得的NAD+和NADH的总量以及NADH的量，即可计算得到样品中NAD+的量以及NAD+/NADH的比值。

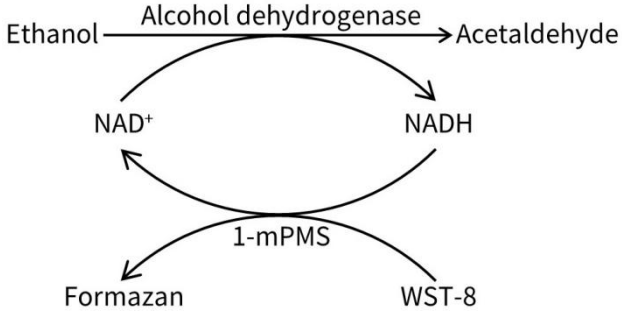

图1. NAD+/NADH检测试剂盒(WST-8法) (S0175)的工作原理图。

- 本试剂盒适用于检测细胞、组织以及其它适当样品中的NAD+和NADH各自的量、比值和总量。
- **本试剂盒提供的NAD+/NADH提取液有一定的通用性**。使用本试剂盒中的NAD+/NADH提取液提取获得的细胞或组织样品，也可以用于碧云天生产的NADP+/NADPH检测试剂盒(WST-8法) (S0179)的检测。
- 在检测组织及其它样品中的NAD+、NADH以及它们的比值时，考虑到有些样品本身的颜色对450nm处吸光值的检测有影响，建

议设置加入样品而不加入乙醇脱氢酶的对照。

- 当仅检测样品中NAD^+和NADH的总量或NADH的量时，一个本试剂盒可以进行100次检测；当检测NAD^+或者NAD^+/NADH的比值时，一个本试剂盒可以进行50次检测。

包装清单：

产品编号	产品名称	包装
S0175-1	乙醇脱氢酶	220μl
S0175-2	显色液	1.1ml
S0175-3	NADH	5mg
S0175-4	NADH配制液	0.8ml
S0175-5	NAD^+/NADH提取液	60ml
S0175-6	反应缓冲液	10ml
—	说明书	1份

保存条件：

-20°C保存，一年有效。显色液(S0175-2)和NADH (S0175-3)须-20°C避光保存。NADH配制成溶液后，须适当分装后-80°C保存。所有试剂避免反复冻融。

注意事项：

- 本试剂盒中的所有试剂均需要冷冻保存，请严格按照保存条件进行保存。如果不是一次用完，为避免反复冻融导致产品失效，请适当分装后保存。
- NADH不太稳定，取出NADH后请尽快使用。如果发现标准曲线不理想，很有可能是标准品发生了降解。
- 由于NAD^+/NADH提取液比较粘稠，以该提取液作为稀释液时，无论对标准品还是样品进行稀释，在稀释过程中务必保证稀释均匀，否则易造成实验数据产生较大波动。
- 细胞或组织样品的提取，请严格按照每100万个细胞或10mg组织加入200μl提取液的比例加入NAD^+/NADH提取液。如果提取液用量过少，得到的样品提取液可能会出现比较粘稠、不易吸取的情况，影响实验操作的便利性，进而影响检测结果的准确性。推荐使用增强型NAD^+/NADH检测试剂盒(WST-8法) (S0176)，可有效解决在提取细胞和组织样品时可能出现的粘稠问题。
- 在样品加样和混匀过程中，须尽量避免产生气泡，以免影响最终的吸光度测定。
- 如果不能非常严格地控制反应温度和反应时间，每次检测都需要设置标准曲线。
- 如果样品溶液中NAD^+和NADH浓度过高或过低，不在试剂盒的线性检测范围内时，可适当调整样品或者提取液的用量。
- 由于NAD^+和NADH很不稳定，在冻存过程中较易降解，所以宜尽量使用新鲜样品进行检测。如果需要进行样品中NAD^+以及NAD^+/NADH比值的测定，在样品加热30分钟以分解NAD^+的过程中，可以先进行样品中NAD^+和NADH的总量的测定，即将NAD^+和NADH的总量的测定和单独NADH的测定分开进行，尽量减少样品在等待过程中因为可能的降解导致的误差。
- 本产品仅限于专业人员的科学研究用，不得用于临床诊断或治疗，不得用于食品或药品，不得存放于普通住宅内。
- 为了您的安全和健康，请穿实验服并戴一次性手套操作。

使用说明：

1. 样品的准备：

a. 细胞样品的准备：对于贴壁细胞，约1×10^6个细胞(大约相当于6孔板一个孔长满的细胞数量)，吸净培养液，按照每100万个细胞加入200μl提取液的比例用移液器加入200μl的NAD^+/NADH提取液，并轻轻吹打，以促进细胞的裂解；对于悬浮细胞，约1×10^6个细胞，600×g离心5分钟，吸净培养液，按照每100万个细胞加入200μl提取液的比例用移液器加入200μl冰浴预冷的NAD^+/NADH提取液，并轻轻吹打，以促进细胞的裂解；裂解过程在室温或冰上操作均可。随后12,000×g，4°C离心5-10分钟，取上清作为待测样品备用。

b. 组织样品的准备：冰上预冷的PBS洗涤组织后，称取约10-30mg的组织样品，用剪刀剪碎，置于匀浆器中，按照每10mg组织加入200μl提取液的比例加入200-600μl的NAD^+/NADH提取液在室温或冰上进行匀浆。随后12,000×g，4°C离心5-10分钟，取上清作为待测样品备用。

注：细胞或组织裂解得到的样品提取液中含有一些酶，可能会消耗NADH。如果希望获得更加理想的检测结果，建议裂解后用超滤管(10kDa)(FUF051)超滤离心，收集超滤后的样品用于后续测试。细胞或组织量较大、提取液用量较少时，得到的样品提取液可能会较为粘稠，导致吸取不便，请严格按照每100万个细胞或10mg组织加入200μl提取液的比例加入NAD^+/NADH提取液进行细胞或组织样品的提取，只要吹打均匀，通常情况下不会出现不便吸取的情况。推荐使用增强型NAD^+/NADH检测试剂盒(WST-8法) (S0176)，可有效解决在提取细胞和组织样品时可能出现的粘稠问题。

2. 试剂盒的准备工作：

a. NADH标准品的配制：吸取655μl NADH配制液，充分溶解本试剂盒提供的5mg NADH后即得到10mM NADH标准品。10mM NADH标准品请适当分装后-80°C避光保存。

b. NADH标准曲线的设置：将10mM的NADH标准品用NAD^+/NADH提取液稀释成适当的浓度梯度，如初次检测可以设置0、0.25、0.5、1、2、4、6、8、10μM这几个浓度，检测时96孔板中每孔加入20μl的标准品，相当于每孔为0、5、10、20、40、80、120、160、200pmol的NADH。如有必要，在后续的实验中可以根据样品中的NADH含量对标准品的浓度范围进行

适当调整。其中浓度为0μM的点为空白对照点，仅含NAD^+/NADH提取液。注意：由于NADH很不稳定，故配制后需尽快使用。

c. 乙醇脱氢酶工作液的配制：将乙醇脱氢酶用反应缓冲液稀释45倍，例如2μl乙醇脱氢酶加入到88μl的反应缓冲液中，即可获得90μl的乙醇脱氢酶工作液。每个标准品或样品的检测需要使用90μl的乙醇脱氢酶工作液，请根据所需检测的标准品和样品的数量，配制适量的乙醇脱氢酶工作液，并注意现配现用。

3. 样品测定：

a. 样品中NAD^+和NADH的总量的测定：吸取20μl用NAD^+/NADH提取液稀释后的待测样品至96孔板中，为了减少实验误差建议设置样品的重复孔。

注：为获得理想的检测效果，建议将细胞样品稀释2-10倍、组织样品稀释5-50倍后进行测定，也可以同时设定多个稀释倍数。后续如果发现样品中的NAD^+和NADH的总量过高，超出标准曲线的范围，则需要加大样品的稀释倍数；总量过低时则需要加大细胞或组织样品的用量或者降低样品的稀释倍数。

b. 样品中NAD^+、NADH 的含量或者NAD^+/NADH比值的测定：吸取50-100μl待测样品于离心管中，60°C水浴或PCR仪上加热30分钟以分解NAD^+。如果加热后产生不溶物，则需10,000×g，室温或4°C离心5分钟，吸取20μl用NAD^+/NADH提取液稀释后的上清液作为待测样品至96孔板中，为了减少实验误差建议设置样品的重复孔。

注：为获得理想的检测效果，建议将细胞样品稀释2-10倍、组织样品稀释5-50倍后进行测定，也可以同时设定多个稀释倍数。后续如果发现样品中的NAD^+或NADH的含量过高，超出标准曲线的范围，则需要加大样品的稀释倍数；含量过低时则需要加大细胞或组织样品的用量或者降低样品的稀释倍数。

c. 请参考下表使用96孔板设置空白对照孔、标准品孔和样品孔。加入乙醇脱氢酶工作液后充分混匀。

	空白对照(Blank)	标准品(Standard)	样品(Sample)
待测样品	—	20μl	20μl
NAD^+/NADH提取液	20μl	—	—
乙醇脱氢酶工作液	90μl	90μl	90μl

d. 37°C避光孵育10分钟。说明：此孵育步骤的目的是将样品中的NAD^+转化为NADH；在加入乙醇脱氢酶工作液的过程中须轻柔操作，以免产生气泡。若不慎出现气泡，可使用细小的吸头或针头戳破。

e. 适当混匀显色液，然后每孔加入10μl显色液，混匀，37°C避光孵育10-20分钟，此时会形成橙黄色的formazan。测量450nm处的吸光度。如果显色较浅，也可以适当延长孵育时间至30-60分钟。

4. 样品中NAD^+/NADH量的计算：

a. 计算标准品组中每个点的平均吸光度，减去空白对照组的吸光度，即为各个标准品的吸光度。

b. 以NADH的浓度为横坐标，吸光度为纵坐标，绘制出标准曲线。NADH标准品的检测效果请参考图2。

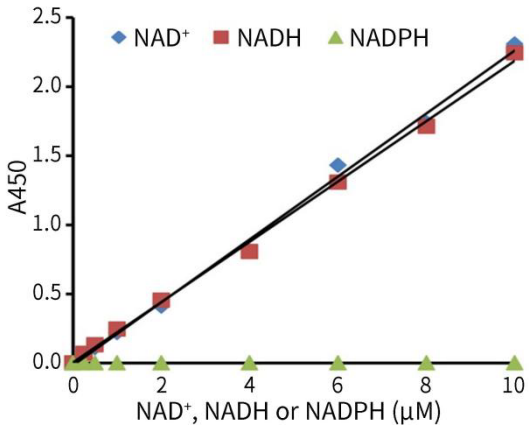

图2. 碧云天NAD^+/NADH检测试剂盒(WST-8法) (S0175)检测NAD^+和NADH的标准曲线。上图显示本试剂盒可以很好地检测出NAD^+和NADH的含量，并且不会受NADPH的干扰。不同的检测条件下，实际读数会因标准品的配制、检测仪器等的不同而存在差异，图中数据仅供参考。

c. 根据标准曲线计算细胞、组织等样品中的NAD^+和NADH总浓度或者NADH的浓度。未60°C加热处理时，检测得到的是样品中NAD^+和NADH总量的浓度(NAD_{total})；60°C加热处理后，检测得到的是样品中NADH的浓度。

备注：根据检测得到的浓度及样品的体积，即可计算出NAD^+、NADH、NAD_{total}的量。

d. 根据如下计算公式，计算样品中NAD^+的量以及NAD^+/NADH的比值。此时可以把NAD^+和NADH总量或各自的含量用单位细胞数量或单位组织重量中的含量来表示。一些细胞和组织中NAD^+和NADH的含量和比值可以参考图3和图4。

$[NAD^+] = [NAD_{total}] - [NADH]$

$[NAD^+]/[NADH] = ([NAD_{total}] - [NADH])/[NADH]$

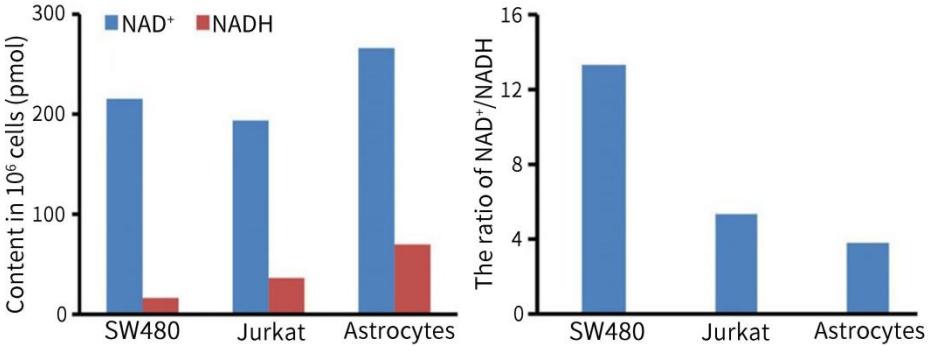

图3. NAD⁺、NADH在一些细胞中的含量和比值(本数据来源于文献或其它资料)。

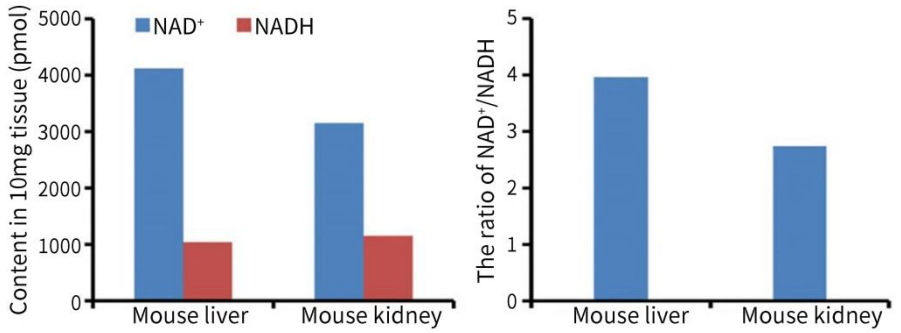

图4. NAD⁺、NADH在小鼠肝脏和肾脏中的含量和比值(本数据来源于文献或其它资料)。

e. 如果希望更加精确地来表述NAD⁺和NADH总量或各自的含量，可以将样品用BCA法测定蛋白浓度。最终用单位蛋白量中NAD⁺和NADH总量或各自的含量来比较精确地进行表述。

相关产品：

产品编号	产品名称	包装
S0175	NAD⁺/NADH检测试剂盒(WST-8法)	100次
S0176S	增强型NAD⁺/NADH检测试剂盒(WST-8法)	100次
S0179	NADP⁺/NADPH检测试剂盒(WST-8法)	100次
S0180S	增强型NADP⁺/NADPH检测试剂盒(WST-8法)	100次
ST358	NADH	100mg
ST360	NADPH	10/50/200mg/1g
ST1110	β-NADPH (≥97%, Reagent grade)	5/25/100mg
ST2213	β-NAD (≥98%, Reagent grade)	250mg/1/5g
ST2218	β-NADH (≥98%, Reagent grade)	250mg/1/5g
ST2812	β-NADP (≥98%, Reagent grade)	50/250mg/1g

使用本产品的文献：

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