Wampole Laboratories®

OSTEOMARK® NTx Urine

试剂盒说明书

酶联免疫吸附法(EIA)定量检测
人尿液中的 I 型胶原交联氨基末端肽(NTx)
仅供体外诊断用

Osteomark 的用途

Osteomark 用于定量测定分泌的交联型 I 型胶原 N 端肽(NTx)。NTx 是骨吸收的一种标记物，尿液 NTx 水平的升高标志着骨吸收水平的升高。NTx 的测量用于下列用途：

A．预测绝经后妇女用激素抗吸收治疗的骨骼反应（骨密度）。

B．治疗监测：

1． 绝经后妇女的抗吸收治疗
2． 诊断为骨质疏松症人群的抗吸收治疗
3． 诊断为佩吉特骨病人群的抗吸收治疗
4． 激素遏制治疗

C．验明补钙和用激素抗吸收治疗的绝经后妇女一年后骨密度降低的相关性

Osteomark 的测量范围是 20~3000nM 骨胶原当量（BCE）。

摘要和试验解释

哺乳动物骨通过破骨细胞介导的骨吸收和随后由成骨细胞介导的骨形成的连续过程，而不断地重塑，这个过程对骨骼的正常发育和维持是必不可少的，这个紧密给合过程的异常往往导致骨量和形状的改变。对特异的骨降解产物的测量，可以为研究骨代谢率提供分析数据。

约 90%的骨组织基质是 I 型胶原，I 型胶原是一种分子 C 端和 N 端交联的螺旋蛋白，这种蛋白组成了骨组织的基本结构和张力。

尿交联型 I 型胶原 N 端肽（NTx）的发现为骨吸收提供了一种物异的生化标记物，这种标记物可以用免疫测定法分析。NTx 分子对骨的专一性基于其氨基酸顺序的独特性和交联的 α_2N 端肽的定位作用。NTx 分子的产生是由骨中破骨细胞介导的，作为一种稳定的降解终产物可存在于尿中。

Osteomark 用于定量测定分泌的 NTx。NTx 作为骨吸收的一种标记物，尿液 NTx 水平的升高标志着骨吸收水平的升高。临床研究表明，骨吸收水平升高是与年龄相关的骨降低的首要原因，低骨量常常导致骨质减少，是骨质疏松症的主要原因[1, 2]，当骨吸收水平高于骨形成水平时骨量降低。据报道，骨质疏松性骨折是老年妇女发病率和死亡率升高的主要因原[3]。

对接受激素替代疗法（HRT）加钙补充（500mg/天），或仅进行钙补充的绝经后妇女进行了一次随机、前瞻性的临床试验。试验结果支持用 Osteomark 预测反应和监测抗吸收治疗的效果[4]。

Osteomark 监测抗吸收治疗的能力，在一项用双磷酸盐(Alendronate sodium,10mg)[5]治疗骨质疏松病人的研究中得到了陈述。结果表明骨质疏松病人在经过三个月的治疗后 Osteomark 值应当被控制在≤35nMBCE/nM 肌酐或者与基准线有 40%以上的改变.。

为了测定 Osteomark 在监测佩吉特骨病病人抗吸收治疗效果的能力，进行了一项研究。Osteomark 基线值和治疗开始后与基线的变化百分率都与血清碱性磷酸酶总量有关。在正常值层面上，Osteomark 在对抗吸收治疗的评估方面早于碱性磷酸酶。抗吸收治疗三个月后，佩吉特病人 Osteomark 离基准值应该有 30%以上的改变。

测定原理

Osteomark 测定是一种竞争抑制酶联免疫吸附测定（ELISA），它用吸附有 NTx 的微孔作固相，标本中的 NTx 和固相中的 NTx 竞争结合辣根过氧化物酶标记的单克隆抗体的结合点，结合到固相上的抗体的量和标本中的 NTx 量成反比。标本中 NTx 的浓度用光度法定量测定，通过一个标准校准曲线计算出来。稀释的尿液测定值用尿液肌酐分析校正，用 nMBCE/mmol 肌酐/L 表示.。

试剂盒组成

所供材料足够 96 人份用

	使用介绍	1 份
A	抗原包被的 96 孔板 12x8 孔条	1 块
B	浓缩抗体结合液	0.4ml 管
C	抗体结合物稀释液	30 ml 瓶
D	30x 浓缩冲洗液	125 ml 瓶
E	显色剂	0.9 ml 瓶
F	缓冲液	30 ml 瓶
G	终止液	25 ml 瓶
1	1 nMBCE 标准品	0.4 ml 管
2	30 nMBCE 标准品	0.4ml 管
3	100 nMBCE 标准品	0.4 ml 管
4	300 nMBCE 标准品	0.4 ml 管
5	1000 nMBCE 标准品	0.4 ml 管
6	3000 nMBCE 标准品	0.4 ml 管
I	尿液质控品 I	0.4 ml 管
II	尿液质控品 II	0.4 ml 管
	板封条	1 包

试剂描述

PLATE	96 孔抗原包被板，12 1x8 条。纯化的人 NTx 抗原吸附到微孔条中（1 块）
CONJ	浓缩抗体结合物，1 管。纯化的鼠抗 NTx 单克隆抗体，结合有辣根过氧化物酶，ProClin (0.05%)作为防腐剂，100 倍浓缩（0.4ml）

CONJ DIL	抗体结合物稀释液，1 瓶。是用于稀释浓缩抗体结合物的缓冲液，ProClin (0.05%)作为防腐剂
WASHBUF 30X	30 倍浓缩冲洗液，1 瓶。离子清洁液。(125 ml)
CHROMOGEN	显色剂，1 管。溶于二甲基亚砜的四甲基联苯胺，100 倍浓缩。（0.9 ml）
BUFFER	基质缓冲液，1 瓶，过氧化氢缓冲液。（30 ml）
SOLN STOP	终止液。1 瓶，1N 硫酸。(25 ml)。注：有些成份中含有牛血清或牛血清白蛋白。
CAL xx nM	分析用标准品：1，30，100，300，1000，3000 nM BCE，各 1 管。纯化的 NTx 抗原溶于缓冲液中，ProClin (0.05%)作为防腐剂。（各 0.4 ml）
CONTROL xx	尿液质控品 I、II。各 1 管，是含已知 NTx 浓度的人的尿液，ProClin (0.05%)作为防腐剂。（各 0.4 ml）

试剂贮存

试剂不用时必须放 2-8 ℃保存，用前平衡至室温，不要将试剂暴露于 30 ℃以上或 2 ℃以下的环境中，稀释后的冲洗液可以在室温存放一个月。

需要但未提供的材料

- 能加样 25μl，100μl 和 200μl 体积的单挡或多挡加样器
- 一次性加样头
- 一次性塑料容器
- 自动洗板机
- 微量板光学读数仪，读数仪能在 450nm 波长和 630nm 参考波长下读数且能测定从 0 到 3.000 的光密度
- 能计算四参数曲线的软件
- 去离子水

尿液标本的收集和贮存

- 收集晨尿标本或 24 小时尿标本，盛在带盖的密闭容器中。
- 尿标本中不要加防腐剂。
- 带血标本或溶血标本可能对测定造成影响，应当弃去，建议重新收集标本。
- 标本冷冻（2-8 ℃）可保存 72 小时，室温保存 24 小时，冰冻（≤20 ℃）可长期保存。标本可经受 3 次冻融。
- 监测治疗时，基准标本应该在治疗开始前收集，后续收集的用于比较治疗效果的标本收集的时间应与基准标本收集的时间一致。

注意事项

- 仅供体外诊断用
- 抗原包被的 96 孔板、标准品和尿液质控品中含有从人尿液和/或骨组织中提呈的抗原，应当按潜在感染品作适当处理。
- 终止液含有 1N 硫酸，不要测到皮肤和眼睛中。一旦被测，立即用水冲洗 15 分钟，测入眼睛时要立即就医。
- 显色剂中含有四甲基联苯胺（TMB）和二甲基亚砜（DMSO）。二甲基亚砜易被皮肤吸收，吸入、接触皮肤或吞咽都会造成伤害，一旦暴露，应立即用流水冲洗暴露部位 15 分钟，如果测入眼中，立即去看医生。
- 尿液标本中可能含有传染原，应作正确外理，用 0.5%的次氯酸钠溶液(家用漂白粉 1:10 稀释)或 121 ℃高压消毒 1 小时，除污效果最佳。不要高压含有次氯酸钠的溶液，也不要将次氯酸钠和酸混合。
- 决不要用嘴吸试剂或临床标本。
- 处理标本和试剂时要穿防护衣，戴防护手套，用完后彻底洗手。
- 不要使用过期试剂。
- 不要将不同批号的试剂混合使用。
- 微孔条必须干燥保存，未使用的板条应当用盛有干燥剂的密封袋重新密封保存。
- 微孔不可重复使用，用后正确处理。
- 测定程序要在符合温育要求的可控实验环境中进行，测试中要避开极端的环境条件。

测定程序

准备步骤：

1. 测定前至少将标本和试剂在室温（18~28℃）平衡 1 小时。为了便于加温，将试剂从试剂盒中取出，冰冻状态的尿标本，可用水浴或温育箱 37℃解冻，然后带至室温。显色剂中含有二甲基亚砜，在冰冻状态是固体，在室温是液体。
2. 准备工作强度冲洗液。将 30X 浓缩冲洗液用去离子水以 1:30 稀释（1 份 30X 浓缩洗涤液＋29 份去离子水）。至少混匀 5 分钟，稀释后的冲洗液在室温可稳定 1 个月。
3. 做一显示标准品、质控品和尿标本位置的板图。建议标准品、质控品和尿标本都做双份，下面是测试 4 份标本的示例：

	1	2	3
A	1 nM 标准品	1000 nM 标准品	1 号标本
B	1 nM 标准品	1000 nM 标准品	1 号标本
C	30 nM 标准品	3000 nM 标准品	2 号标本
D	30 nM 标准品	3000 nM 标准品	2 号标本
E	100 nM 标准品	质控品 I	3 号标本
F	100 nM 标准品	质控品 I	3 号标本
G	300nM 标准品	质控品 II	4 号标本
H	300nM 标准品	质控品 II	4 号标本

4. 在一个干净一次性使用塑料容器中，用抗体结合物稀释液将浓缩抗体结合物以 1:101 的比例稀释。参照；每条抗原包被的微孔条，需用 2ml 抗体结合物稀释液稀释 20μl 浓缩抗体结合物，轻轻旋转混匀，不要形成涡流，

也不要使用磁搅拌棒，以免形成泡沫。工作强度结合物液 1 小时内使用，容器不可重复使用。

5. 测定前轻轻混匀标准品、质控品和尿液标本，不要形成泡沫。云雾状或混浊标本放置 5-10 分钟再吸样，含颗粒尿标本用前可离心。
6. 从密封锡箔中取出需要数量的微孔条，剩余部分放回密封袋，沿拉链密封，干燥剂要保留。

标本和抗体的温育：

测定一旦开始，请不要间断。

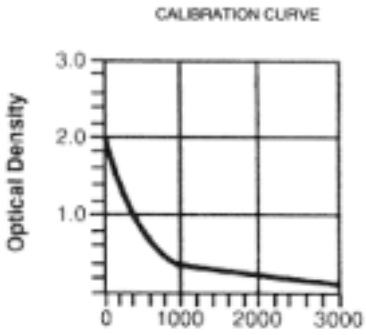
7. 根据步骤 3 中板的布局，各加 25µl 的标准品、质控品、尿液标本于相应的微量板孔中。加样器要经过校准，每次加样后都要换用一个新吸头。
8. 用多挡加样器向每个加样孔中加入 200µl 工作强度结合物液，盖密封条，在平面上将板轻轻混匀 5-10 秒钟。
9. 在室温（18~28ºC）温育 90±5 分钟。
10. 在温育的最后 10 分钟准备显色剂/基质缓冲液。用基质缓冲液将显色剂以 1:101 的比例稀释。参照：每条抗原包被条需要用 2ml 基质缓冲液稀释 20µl 显色剂。将基质缓冲液加到一个一次性塑料容器中，将显色剂充分混匀后加入到基质缓冲液中，轻转混匀，不要巨烈摇动或使用磁搅拌棒。配制的显色剂/基质缓冲液要在 30 分钟内使用。混匀后溶液应当是无色的，兰色表明试剂被污染，应当弃去。配制显色剂/基质缓冲液的容器不可再用。
11. 温育结束，小心揭去密封条，在自动洗板机上用工作强度冲洗液洗孔 5 次，每个冲洗循环每孔最少用 350µl 冲洗液。冲洗过程中每孔中应注满工作强度冲洗液。冲洗完毕，抓住板的四周中部,倒转,在吸水纸上拍干。立即按下所述加入显色剂/基质缓冲液。

显色和测量：

12. 用一多挡加样器向每孔中加入 200µl 步骤 10 中准备的显色剂/基质缓冲液，盖一新封条。
13. 在室温温育 15±1 分钟，结合有辣根过氧化物酶标记抗体的孔中会有兰色出现。
14. 温育结束，小心揭去封条。用一多挡加样器按加显色剂/基质缓冲液同样的顺序向每孔中加入 100µl 终止液，兰色孔中颜色将变为黄色。
15. 在平面上将板轻轻旋转混匀 5-10 秒钟，将板在室温放置 5 分钟后测吸光度值。
16. 加终止液后 30 分钟内，用微量读数仪读取标准品、质控品和尿液标本的吸光度（波长 450nm,参考波长 630nm）。读数仪的最大光度读数应当≥3.000。

结果分析

1. 用四参数对数曲线固定方程作出标准曲线测定质控品和尿液标本的浓度值（nM BCE）。



例如：

2. 结果在满足下列条件的情况下是有效的：
   - 1nMBCE 标准品的吸光度均值必须≥1.500
   - 标准曲线的范围(1 nM BCE 标准品和 40 nM BCE 标准品的吸光度差异)必须≥1.300
3. 推荐的双份测定尿标本浓度值的变异系数(%CV)是≤20%，变异系数>20%的样本应当重测。
4. 测定低限是 20 nM BCE（不包括肌酐调整值）。
5. 超过 3000nMBCE 的尿标本可以用 200-500 nM BCE 范围的尿标本 1:5 稀释后重测。当用尿液做稀释液时，稀释液的 nM BCE 值也在同一板块中以同样的方式测定出来。稀释因子和背景（稀释液的 nMBCE）也要合并计算。

例如：用 Osteomark 值是 300nMBCE 的尿液做稀释液，以 1:5 的比例稀释 4000nMBCE 的标本，测得值为 1040 nM BCE

1040 nM BCE-(0.8×300 nM BCE)= 800 nM BCE
800nMBCE×5(稀释因子)= 4000 nM BCE
注：1:5 稀释表示 80%（0.8）是稀释液，20%是标本。

6. 尿标本的报告浓度值为 nMBCE/mM 肌酐，如下例所示：
测定值 = 360nMBCE
尿肌酐值 = 60mg/dl 肌酐÷11.3* = 5.3mM 肌酐
300nMBCE÷5.3mM 肌酐 = 68nMBCE/mM 肌酐
*注：将毫克每升转化为毫摩尔每升的转化因子。
7. 制造商已经建立了尿液质控范围，建议每个试验室确立自己的质控范围。

局限性

虽然 Osteomark 被用作骨吸收的标记物，但尚未用于预测骨质疏松症的形成和未来发生骨折的风险，也未用于诊断原发性甲状旁腺机能亢进、甲状腺机能亢进。当用 Osteomark 监测治疗效果时，可能会受到影响骨吸收的其他临床条件的影响，例如：骨转移，然而 Osteomark 提供了一种检测骨吸收水平的方法。由于报告结果不包含时间因素，单一的 Osteomark 值不能提供骨吸收率。Osteomark 结果应当结合临床和其他诊断结果综合分析。

干扰物质

对尿液中各种可能对 Osteomark 测定造成干扰的成份和微生物进行了评估。已检测微生物，如：大肠埃希菌（ATCC

25922)、铜绿假单胞菌（ATCC 27853)、白色假丝酵母菌（ATCC 14053）都对测定无干扰，人白蛋白、胆红素、糖和维生素 C 对测定也无干扰。血尿标本和溶血标本可能对测定造成干扰，应当弃去，重留。

预期值

为了确立停经前妇女（年龄 25~49 岁，平均年龄 36 岁）和男子（年龄 31~87 岁，平均年龄为 56 岁）的参考范围进行了一项多重交叉研究。

表 1：男子和停经前妇女 Osteomark 值预期范围

	均值*	标准差	范围(均值±SD)*	N
妇女	35	15	5 - 65	258
男子	33	15	3 - 63	206
*以 nMBCE/mM 肌酐表示				

停经前妇女的预期值范围经对数转换后变为 14-74nMBCE/mM 肌酐，经对数转换后的男性参考范围为 13-78nMBCE/mM 肌酐。

从对健康绝经后妇女(n=276，268)和 30 岁以上的男人(n=36，35）的前瞻性研究中确立了短期和长期变异。连续收集 3-4 天尿标本测定短期变异，连续收集 2-3 个月尿标本测定长期变异。短期变异：妇女是 15%，男子是 18%。长期变异：妇女是 18%，男子是 19%。

操作特性

重复性和精密度

批内测定变异是由 4 个操作者分别对 8 份尿液标本重复测定 10 次而评估的。结果如下：

标本	均值(nMBCE)	变异系数(%)
A	26	19
B	111	8
C	172	8
D	417	5
E	694	5
F	1113	5
G	1768	6
H	2640	5

批间测定变异是由一个操作者对 3 份尿标本重复测定 20 次而进行评估的。结果如下：

标本	均值(nMBCE)	变异系数(%)
A	79	5
B	412	3
C	1167	4

总测定精密度是通过 30 天中在 3 家临床实验室测定尿液质控品 I 和尿液质控品 II 进行评价的。尿液质控品 I 的均值为 439 nMBCE，变异系数为 10%，尿液质控品 II 的均值是 1537 nMBCE，变异系数为 7%。

抗原回收

抗原回收是通过向 3 份已知 NTx 浓度的尿标本中加入已知量的 NTx 来评价的。回收表示测得值占理论值（尿液基准值加新加入的抗原(NTx)值）的百分比。结果表明抗原回收率是 105%。

稀释线性

稀释线性是通过对 4 份高 nMBCE 的尿液标本用 200-500nMBCE 的尿液标本做系列稀释来评价的。结果显示相关系数 r=0.999 或 r=1.000。

临床研究

Osteomark 在绝经后妇女中的应用

为了测定 Osteomark 测定在监测激素替代疗法（HRT）对骨吸收的影响方面的能力，以及预测对 HRT 的反应，对绝经早期妇女进行了一项试验。结果支持对绝经早期妇女用 Osteomark 监测激素抗吸收治疗，以及预测骨密度（BMD）对 HRT 的反应性改变，这种改变可以用双能量 X 线吸收仪测定。因此，象测量骨密度一样，可以确定谁将从这种治疗中获得最大益处，以及谁有骨量降低的风险。治疗组和对照组的四项对比检查提供了 BMD 降低的风险信息（表 2）：在最低 NTx 四分位限(≤38nMBCE/mM 肌酐)，HRT 组和对照组 1 年后骨质降低的可能性无显著性差别，高基准值 NTx(>67nMBCE/mM 肌酐)，在不用 HRT 治疗时，风险增长了 17.3 倍。

表 2.比较钙组和 HRT 组 BMD 降低的相对风险		
NTx*基准值(nMBCE/m 肌酐)	相对风险	95%C.I
18 - 38	1.4	0.8-2.5
38 - 51	2.5	1.0-6.1
51 - 67	3.8	1.6-9.1
67 - 188	17.3	2.5-118.5
*N=54，53，57，55		

Osteomark 在诊断为骨质疏松病人中的应用

进行了一项研究来确立一种新药氨基双磷酸（alendronate）在治疗骨质疏松症方面的安全性和功效。下列资料支持 Osteomark 用于监测因患骨质疏松症而抗吸收治疗(alendrote 10mg)的妇女的骨吸收水平。

- 治疗三个月后，80%（71/89）的受试者 Osteomark 值≤35 nMBCE/mM 肌酐，87%（76/87）的受试者有 40%以上的降低。
- 治疗一年后，90%（77/86）的受试者 Osteomark 值≤35 nMBCE/mM 肌酐，92%（77/84）的受试者有 40%以上的降低。
- 因此，患骨质疏松症抗吸收治疗后妇女的 Osteomark 值，三个月后应当≤35 nMBCE/mM 肌酐，或有 40%以上的降低。

Osteomark 在诊断为佩吉特骨病的病人中的应用

进行了一项研究来测定 Osteomark 监测佩吉特骨病病人用双磷酸盐治疗后影响骨吸收的能力。以下资料支持 Osteomark 用于监测佩吉特骨病病人采用双磷酸盐治疗对骨

吸收水平的影响。

时间	Alendronate	Etidronate	Pamidronate
1 个月*	-48%	-39%	-71%
3 个月**	-77%	-58%	-67%
6 个月***	-87%	-72%	-71%

*N=22，17，20
**N=28，23，20
***N=27，22，20

*抗吸收治疗佩吉特骨病病人的 Osteomark 值应当被监测至有 30%以上的改变或回至正常范围。

表 4. 标准标记物的应答百分率（Osteomark 5-65nMBCE/mM 肌酐，总碱性磷酸酶 39-115 IU/L；标记物值病人数目/总病人数目）

时间 *	Osteomark 应答者	总碱性磷酸酶应答者
1 个月	19%	2%
3 个月	34%	28%
6 个月	42%	42%

*N=59，71，69

总碱性磷酸酶降低至正常范围作为治疗有效的指标，Osteomark 值也用于应答，并且早于总碱性磷酸酶的评估价值。

Osteomark 在抑制雌激素治疗中的应用

对绝经前妇女进行了一项临床试验来测定 Osteomark 对监测雌激素抑制治疗对骨吸收的影响的测定能力 [6]。结果支持 Osteomark 用于监测雌激素抑制治疗对骨吸收水平的影响。

参考文献

略，请参见原文说明书

Osteomark NTx Urine 快速参考

1. 先将测定程序通读。
2. 把标本和试剂放至室温。
3. 用抗体结合物稀释液将浓缩抗体结合物以 1:101 比例稀释，轻轻颠倒混匀，新配制的工作强度结合物液 1 小时内使用。
4. 各加 25µl 标准品、质控品和尿液标本于相应的微孔中。
5. 每孔中加入 200µl 工作强度结合物液，轻轻混匀，在室温中温育 90±5 分钟。
6. 在温育的最后 10 分钟准备显色剂/基质缓冲液，将显色剂用基质缓冲液以 1:101 的比例稀释，轻轻颠倒混匀，不要剧烈晃动，也不要用磁搅拌器，配制后 30 分钟内使用。
8. 用工作强度冲洗液以每孔 350µl 的量洗板 5 次，在吸水纸上拍干。
9. 每孔中加 200µl 显色剂/基质缓冲液，室温温育 15±1 分钟。
10. 每孔中加 100µl 终止液，轻轻旋转混匀。
11. 室温温育 5 分钟后，在 450nm-630nm 波长下读取每孔吸光度值。用四参数曲线固定方程计算结果。

重要提示：
本中文译稿仅供参考。
开始试验前务必详细阅读试剂盒所附英文说明书，
并严格按照英文说明书进行操作。谨防有误。

Wampole Laboratories®

OSTEOMARK® NTx Urine

An Enzyme-linked Immunosorbent Assay (EIA) for the Measurement of Cross-linked N-telopeptides of Type I Collagen (NTx) in Human Urine

For *in vitro* diagnostic use only

Intended Use of Osteomark

Osteomark is a urinary assay that provides a quantitative measure of the excretion of cross-linked N-telopeptides of type I collagen (NTx) as an indicator of human bone resorption. Elevated levels of urinary NTx indicate elevated human bone resorption. Measurement of NTx is intended for use in:

A. Predicting skeletal response (bone mineral density) to hormonal antiresorptive therapy in postmenopausal women
B. Therapeutic monitoring of:
1. antiresorptive therapies in postmenopausal women
2. antiresorptive therapies in individuals diagnosed with osteoporosis
3. antiresorptive therapies in individuals diagnosed with Paget's disease of bone
4. estrogen-suppressing therapies

C. Identifying the probability for a decrease in bone mineral density after one year in postmenopausal women receiving calcium supplement relative to those treated with hormonal antiresorptive therapy.

The measurement range of Osteomark is 20 to 3000 nM Bone Collagen Equivalents (BCE).

Summary and Explanation of the Test

Mammalian bone is continuously remodeled through a coupled process of osteoclast mediated bone resorption, followed by osteoblast mediated bone formation. This process is necessary for normal development and maintenance of the skeleton. Abnormalities in this tightly coupled process often result in changes in skeletal mass and shape. The measurement of specific degradation products of bone matrix provide analytical data of the rate of bone metabolism.

Approximately 90% of the organic matrix of bone tissue is type I collagen. Type I collagen, a helical protein that is cross-linked at the N-terminal and C-terminal ends of the molecule, forms the basic fabric and tensile strength of bone tissue.

The discovery of urinary cross-linked N-telopeptides of type I collagen (NTx) has provided a specific biochemical marker of human bone resorption which can be analyzed by immunoassay. The NTx molecule is specific to bone due to the unique amino acid sequences and orientation of the cross-linked alpha-2 (I) N-telopeptide. Generation of the NTx molecule is mediated by osteoclasts on bone, and is found in the urine as a stable end-product of degradation.

Osteomark provides a quantitative measure of the excretion of NTx as an indicator of human bone resorption. Elevated levels of urinary NTx indicate elevated bone resorption. Clinical research has demonstrated that elevated bone resorption is the primary cause of age-related bone loss and that low bone mass often results in osteopenia and is the major cause of osteoporosis.[1, 2] Loss of bone mass occurs when bone resorption levels are elevated above bone formation levels. Osteoporotic fractures are reported to be the major source of increased morbidity and mortality in older women.[3]

A randomized, prospective clinical trial of postmenopausal women treated with either hormone replacement therapy (HRT) plus calcium supplements (500 mg daily) or calcium supplements alone, supported the use of Osteomark to predict response and monitor the antiresorptive effect of the therapy.[4]

The ability of Osteomark to monitor the effect of antiresorptive therapy has been demonstrated in a study of patients diagnosed with osteoporosis treated with a bisphosphonate (alendronate sodium, 10 mg).[5] Results demonstrate that the osteoporotic patient should be managed to an Osteomark value of ≤35 nM BCE/mM creatinine or have a ≥40% change from baseline after 3 months of treatment.

A study was conducted to determine the ability of Osteomark to monitor the effect of antiresorptive therapy in patients with Paget's disease of bone. Osteomark values at baseline and the percent change from baseline after therapy initiation correlated to serum total alkaline phosphatase. Osteomark provided an earlier assessment than alkaline phosphatase of therapeutic response, defined by normalization of values. Paget's patients treated with antiresorptive therapy should have a ≥30% change in Osteomark from baseline after 3 months of treatment.

Assay Principles

The Osteomark assay is a competitive-inhibition enzyme-linked immunosorbent assay (ELISA) that utilizes microwells as the solid phase onto which NTx has been adsorbed. NTx in the specimen competes with the solid phase NTx for binding sites of a monoclonal antibody labeled with horseradish peroxidase. The amount of antibody bound to the solid phase is therefore inversely proportional to the amount of NTx in the specimen. Quantitation of the NTx concentration in the specimen is determined spectrophotometrically and calculated from a standard calibration curve. Assay values are corrected for urinary dilution by urinary creatinine analysis and expressed in nanomoles bone collagen equivalents per liter (nM BCE) per millimole creatinine per liter (mM creatinine).

Kit Components
Supplied Materials Sufficient for 96 wells

	Instructions for Use (1 booklet)	1 booklet
A	Antigen Coated 96-well Plate, 12 1x8-well strips	1 Plate
B	Antibody Conjugate Concentrate	0.4 mL vial
C	Antibody Conjugate Diluent	30 mL bottle
D	30X Wash Concentrate	125 mL bottle
E	Chromogen Reagent	0.9 mL bottle
F	Buffered Substrate	30 mL bottle
G	Stopping Reagent	25 mL bottle
1	1 nM BCE Calibrator	0.4 mL vial
2	30 nM BCE Calibrator	0.4 mL vial
3	100 nM BCE Calibrator	0.4 mL vial
4	300 nM BCE Calibrator	0.4 mL vial
5	1000 nM BCE Calibrator	0.4 mL vial
6	3000 nM BCE Calibrator	0.4 mL vial
I	Level I Urine Control	0.4 mL vial
II	Level II Urine Control	0.4 mL vial
	Plate Sealers	1 pad

Reagent Description

PLATE	Antigen Coated 96-Well Plate, 12 1x8 well strips. Purified human NTx antigen adsorbed onto microwell strips. (1 plate)
CONJ	Antibody Conjugate Concentrate, 1 vial. Purified murine monoclonal antibody directed against NTx and conjugated to horseradish peroxidase. ProClin™ 300 (0.10%) is included as a preservative. Supplied as a 100X concentrated reagent. (0.4 mL)
CONJ DIL	Antibody Conjugate Diluent, 1 bottle. Buffered reagent with protein stabilizers, into which Antibody Conjugate Concentrate is diluted. ProClin™ 300 (0.05%) included as a preservative. (30 mL)
WASHBUF 30x	30X Wash Concentrate, 1 bottle. Ionic detergent solution. Supplied as 30X concentrate. (125 mL)
CHROMOGEN	Chromogen Reagent, 1 vial. 3,3',5,5' – tetramethylbenzidine in dimethyl-sulfoxide. Supplied as a 100X concentrated reagent. (0.9 mL)
BUFFER	Buffered Substrate, 1 bottle. Buffered hydrogen peroxide. (30 mL)
SOLN STOP	Stopping Reagent, 1 bottle. 1N sulfuric acid. (25 mL) Note: Bovine Serum or Bovine Serum Albumin is present in some components.
CAL xx nM	Assay Calibrators: 1, 30, 100, 300, 1000, 3000 nM BCE, 1 vial each. Purified NTx antigen in buffered diluent. ProClin™ 300 (0.05%) included as a preservative. (0.4 mL each)
CONTROL xx	Level I and Level II Urine Controls, 1 vial each. Human urine with known NTx concentration. ProClin™ 300 (0.10%) included as a preservative. (0.4 mL each)

Storage of Reagents
Reagents must be stored at 2 - 8 °C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 30 °C or less than 2 °C. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required but Not Supplied
- Single and multichannel pipettes capable of delivering 25 µL, 100 µL and 200 µL volumes.
- Disposable pipet tips.
- Disposable plastic containers for reagent mixing and pipetting reservoirs.
- Automated microwell washer.
- Microwell or microstrip spectrophotometric reader. The reader must read at 450nm with a 630 nm reference filter and detect absorbances from 0 to 3.000 optical density units.
- Software capable of calculating a 4-parameter curve fit.
- Deionized water.

Urine Specimen Collection and Storage
- Collect a second void of the morning (spot) urine specimen or a 24 hour urine specimen in an appropriate collection device with a tight fitting lid.
- DO NOT ADD PRESERVATIVE TO URINE SPECIMEN.
- Specimens with visible whole blood contamination or visible hemolysis may interfere with the assay and should be discarded. Collection of a new specimen is recommended.
- Store refrigerated (2 - 8 °C) for up to 72 hours or at room temperature for up to 24 hours. Store frozen (–20 °C or below) for longer term storage. Specimens may undergo three freeze/thaw cycles.
- **When monitoring therapy, baseline samples should be collected prior to initiation of therapy. Subsequent specimens for comparison should be collected at the same time of day as the baseline specimen.**

Warnings and Precautions

- **For in vitro diagnostic use only**
- The Antigen Coated 96-Well Plate, Calibrators, and Urine Controls contain human urine and/or processed antigen from human bone tissue and therefore should be handled as potentially infectious materials and disposed of appropriately.
- The Stopping Reagent contains 1N sulfuric acid. Avoid contact with skin or eyes. If exposed, flush area with water for 15 minutes. If eyes are exposed, obtain medical attention.
- The Chromogen Reagent contains 3,3',5,5'- tetramethylbenzidine and dimethylsulfoxide. Dimethylsulfoxide is readily absorbed through the skin. This reagent is harmful by inhalation, contact with skin, or if swallowed. If exposed, flush area with water for 15 minutes. If eyes are exposed obtain additional medical attention.
- Urine specimens may contain infectious agents and should be disposed of properly. Decontamination is most effectively accomplished with a 0.5% solution of sodium hypochlorite (1:10 dilution of household bleach) or by autoclaving one hour at 121 °C. Do not autoclave solutions containing sodium hypochlorite. Do not combine sodium hypochlorite solution with acid.
- Never pipette reagents or clinical specimens by mouth.
- Wear protective gloves and clothing when handling specimens and reagents. Wash hands thoroughly after use.
- Do not use reagents beyond their expiration dates.
- Do not mix components from different lots of Osteomark kits.
- Microwell strips must be kept desiccated. Reseal unused microwell strips in the
- pouch containing desiccant.
- Do not reuse microwells. Dispose of properly after use.
- Perform the assay procedure in a controlled laboratory environment that adheres
- to the stated incubation requirements. Avoid extreme environmental conditions
- during the procedure.

Assay Procedure

Preparatory Steps

1. **Allow all specimens and reagents to equilibrate to room temperature (18 - 28 °C) for at least one hour before performing the assay.** To facilitate warming, remove reagents from the kit box. Frozen urine specimens may be thawed at 37 °C, in either a water bath or an incubator, then brought to room temperature prior to use in the assay. The Chromogen Reagent contains dimethylsulfoxide, which may solidify when refrigerated but is liquid at room temperature.
2. Prepare the working strength wash solution. Dilute 30X Wash Concentrate 1:30 with deionized water (1 part 30X Wash Concentrate to 29 parts deionized water) and mix for a minimum of five (5) minutes. This solution is stable for one (1) month at room temperature.
3. Create a plate map indicating location of calibrators, controls and urine specimens. It is recommended that calibrators, controls and urine specimens be run in duplicate microwells. An example of a plate map is provided below for an Osteomark assay with 4 specimens:

	1	2	3
A	1 nM BCE Calibrator	1000 nM BCE Calibrator	Specimen #1
B	1 nM BCE Calibrator	1000 nM BCE Calibrator	Specimen #1
C	30 nM BCE Calibrator	3000 nM BCE Calibrator	Specimen #2
D	30 nM BCE Calibrator	3000 nM BCE Calibrator	Specimen #2
E	100 nM BCE Calibrator	Level I Urine Control	Specimen #3
F	100 nM BCE Calibrator	Level I Urine Control	Specimen #3
G	300 nM BCE Calibrator	Level II Urine Control	Specimen #4
H	300 nM BCE Calibrator	Level II Urine Control	Specimen #4

4. Using a clean disposable plastic container, dilute the Antibody Conjugate Concentrate 1:101 using the Antibody Conjugate Diluent. As a guideline, for each Antigen Coated microwell strip used, dilute 20 µL Antibody Conjugate Concentrate into 2 mL of Antibody Conjugate Diluent. Mix gently by inversion only. **Do not vortex or use a magnetic stir bar.** Avoid foaming. Use the working strength conjugate solution within one hour of preparation. Do not reuse the container.
5. Prior to pipetting, gently mix the Calibrators, Controls and urine specimens. Avoid foaming. Allow cloudy or turbid specimens to settle 5 to 10 minutes prior to pipetting. Urine specimens containing particulates may be centrifuged before use.
6. Remove the appropriate number of microwell strips from the sealed foil pouch. Place any unused strips back in the pouch, resealing the pouch along the zipper. Do not remove the desiccant pillow from the foil pouch.

Specimen and Antibody Incubation

Once the assay has been started, complete it without interruption.

7. Following the plate map generated in Step 3, pipette 25 µL of each Calibrator, Control, or urine specimen into the bottom of the designated microwells. Use a calibrated pipettor and new pipette tips for each Calibrator, Control, or urine specimen.
8. Using a multichannel pipettor, deliver 200 µL of the working strength conjugate solution into each microwell. Apply a plate sealer and swirl the plate gently on a flat surface for 5-10 seconds to ensure mixing.
9. Incubate the plate at room temperature (18 - 28 °C) for 90 ± 5 minutes.
10. Prepare the Chromogen/Buffered Substrate solution during the last 10 minutes of incubation by making a 1:101 dilution of the Chromogen Reagent into the Buffered Substrate. As a guideline, for each Antigen Coated microwell strip used, dilute 20 µL of the Chromogen Reagent into 2 mL of the Buffered Substrate. Pipette the Buffered Substrate into a clean plastic disposable container. Thoroughly mix the Chromogen Reagent prior to pipetting. Add the Chromogen Reagent to the Buffered Substrate Reagent and invert gently to mix. **Do not vortex, shake vigorously or use a magnetic stir bar to mix. Use the Chromogen/Buffered Substrate solution within 30 minutes of preparation.** The Chromogen/Buffered Substrate solution should be colorless when mixed. A blue color indicates that the reagent has been contaminated and must be discarded. **Do not reuse the Chromogen/Buffered Substrate solution container**.
11. At the end of the incubation period, carefully remove and discard the plate sealer. Wash the plate five (5) times with the working strength wash solution using an automated plate washer. The automated washer must dispense at least 350 µL of the working strength wash solution per well. Between wash cycles, wells should be filled with working strength wash solution. When wash procedure is complete, grasp the plate frame at the center of each side and invert, blotting on an absorbent paper towel. Immediately add the prepared Chromogen/Buffered Substrate solution as described below.

Color Development and Measurement

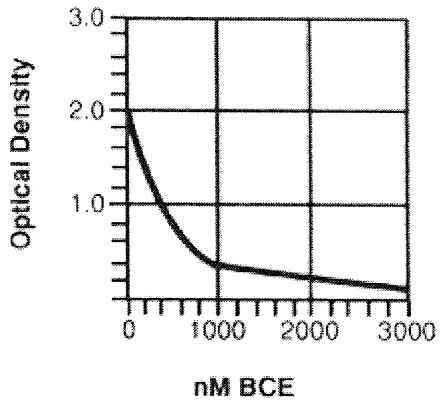
12. Pipet 200 µL of the Chromogen/Buffered Substrate solution prepared in step 10 into each well using a multichannel pipettor. Cover the plate with a new plate sealer.
13. Incubate at room temperature for 15 ± 1 minutes. A blue color will develop in wells containing bound antibody-horseradish peroxidase conjugate.
14. Following incubation, carefully remove and discard the plate sealer. Using a multichannel pipettor, add 100 µL of Stopping Reagent to each well in the same order as addition of the Chromogen/Buffered Substrate Reagent. Wells which have developed a blue color will now turn yellow.
15. Swirl the plate gently on a flat surface for 5 -10 seconds to ensure mixing. Allow the plate to sit at room temperature for 5 minutes before reading absorbance values.
16. Within 30 minutes of adding the Stopping Reagent, read the absorbance of the Calibrators, Controls, and urine specimens. Use a microwell plate reader at 450 nm with a reference filter of 630 nm. The reader must have a maximum optical density reading of ≥3.000.

Analysis of Results

1. Determine concentration values (nM BCE) of Controls and urine specimens using a 4-parameter curve fitting equation.

EXAMPLE:

CALIBRATION CURVE



2. **Assay results are valid if the following criteria are met:**
   - **The mean absorbance value of the 1 nM BCE Calibrator must be ≥1.500.**
   - **The span of the calibrator curve (difference between absorbance values of the 1 nM BCE and 3000 nM BCE Calibrators) must be ≥1.300.**
3. **The recommended coefficient of variation (% CV) between urine specimen concentration value (nM BCE) duplicates is ≤20%. Specimens with >20% CV should be rerun.**
4. The lower limit of detection is 20 nM BCE (assay value, does not include creatinine correction).
5. Urine specimens that exceed 3000 nM BCE may be diluted 1:5 in a urine specimen or pool of urine known to be within the range of 200-500 nM BCE, and retested. When using urine as a diluent, the nM BCE of the urine diluent should be confirmed by testing it as a specimen in the same plate as the diluted unknown specimen. The dilution factor and background (the diluent nM BCE) should be incorporated into the final calculation.

   Example: 1040 nM BCE assay value derived from a 1:5 dilution of a 4000 nM BCE specimen using a urine diluent of known Osteomark value (300 nM BCE)

   1040 nM BCE - (0.8 x 300 nM BCE) = 800 nM BCE
   800 nM BCE x 5 (dilution factor) = 4000 nM BCE

   Note: 1:5 dilutions represent 80% diluent (0.8), 20% specimen contribution.

6. Report the concentration values for urine specimens as nM BCE/mM creatinine, as shown I the following example:

Assay value	=	360 nM BCE
Urinary creatinine	=	60 mg/dL creatinine / 11.3*
	=	5.3 mM creatinine
300 nM BCE / 5.3 mM creatinine	=	68 nM BCE/mM creatinine

*Note: Conversion factor used to convert mg creatinine per dL to millimole creatinine per liter.

7. These Urine Control ranges have been established by the manufacturer. It is recommended that each laboratory establish its own control ranges

Limitations of the Procedure

While Osteomark is used as an indicator of bone resorption, use of this test has not been established to predict development of osteoporosis or future fracture risk. Use of this test has not been established in primary hyperparathyroidism or hyperthyroidism. When using Osteomark to monitor therapy, results may be confounded in patients afflicted with clinical conditions known to affect bone resorption, e.g., metastases to bone. While an Osteomark value provides a measure of the level of bone resorption, a single Osteomark value cannot

provide the rate of bone resorption as reported results do not contain a measure of time. Osteomark results should be interpreted in conjunction with clinical findings and other diagnostic results.

Interfering Substances
Various urine components and microorganisms were evaluated for an interfering effect in the Osteomark assay. The organisms tested, E. coli (ATCC 25922), P. aeruginosa (ATCC 27853), and C. albicans (ATCC 14053), did not interfere with assay performance. Human albumin, bilirubin, glucose, and vitamin C did not interfere with assay performance. Urine specimens obviously contaminated with whole blood or that have visible hemolysis may interfere with assay performance. These specimens should be discarded, and a new urine specimen collected.

Expected Values
A multi-center, cross-sectional study was conducted to determine the reference range for normal premenopausal women (mean age 36 years, range 25-49) and men (mean age 56 years, range 31-87).

Table 1:
Expected Osteomark Values for Men and Premenopausal Women Range

	Mean*	Std Dev	Range (mean ± std dev)*	N
Women	35	15	5-65	258
Men	33	15	3-63	206
* expressed as nM BCE/mM creatinine				

When the premenopausal women expected value range is log-transformed, the range is 14-74 nM BCE/mM creatinine. The log-transformed reference range for men is 13-78 nM BCE/mM creatinine.

The expected short- and long-term intra-subject variability was determined from prospective studies conducted in healthy postmenopausal women (n=276, 268) and men greater than 30 years of age (n=36, 35). Short-term variability was determined from sequential urine specimens collected over 3-4 days and long-term variability was determined from 2-3 monthly collections. Short-term variability was 15% for women and 18% for men. Long-term variability was 18% for women and 19% for men.

Performance Characteristics
Reproducibility and Precision
Intra-assay variability was assessed using eight urine specimens tested in replicates of 10 by each of four operators. Results are provided below:

Sample	Mean (nM BCE)	CV (%)
A	26	19
B	111	8
C	172	8
D	417	5
E	694	5
F	1113	5
G	1768	6
H	2640	5

Inter-assay variability was assessed using three urine specimens tested in duplicate by one operator over 20 separate assay runs. Results are provided below:

Sample	Mean (nM BCD)	CV %
A	79	5
B	412	3
C	1167	4

Total assay precision was evaluated by testing the Level I Urine Control and the Level II Urine Control at three clinical laboratory sites over a 30 day period. The Level I Urine Control had a mean value of 439 nM BCE, with a 10% CV. The Level II Urine Control had a mean value of 1537 nM BCE, with a 7% CV.

Antigen Recovery
Antigen recovery was evaluated by adding known amounts of NTx to each of three urine specimens of known NTx concentration. Recovery represented the observed assay value of the "spiked" specimens, calculated as a percent of the expected urine value (baseline urine value plus added antigen (NTx) value). The results demonstrated an average antigen recovery of 105% across the assay range.

Dilutional Linearity
Dilutional linearity was evaluated by performing serial dilutions of four urine specimens with high nM BCE values into a urine specimen with a 200-500 nM BCE value. Results demonstrated correlation coefficients of r = 0.999 to r = 1.000 across the assay range.

Clinical Studies
Use of Osteomark in Postmenopausal Women
A clinical trial was conducted to determine the ability of the Osteomark assay to monitor the effect of hormone replacement therapy (HRT) on bone resorption in early postmenopausal women, and to predict response to HRT. Results support the clinical utility of Osteomark to monitor hormonal antiresorptive therapy in early postmenopausal women and to predict changes in bone mineral density (BMD) measured by dual energy x-ray absorptiometry (DEXA) in response to HRT, thereby identifying who will receive the greatest benefit from such therapy, and to identify those at risk for bone loss as measured by BMD.4 Examination of the contrast between treated and control groups provides information regarding risk of BMD loss (Table 2): In the lowest NTx quartile (≤38 nM BCE/mM creatinine), there was no statistically significant difference between the HRT and control groups in the

likelihood of bone loss over 1 year. A high baseline NTx (>67 nM BCE/mM creatinine) indicated a 17.3 times higher risk of BMD loss if not treated with HRT.

Table 2.
Relative Risk of Loss of BMD Comparing Calcium and HRT Groups

Baseline NTx* (nM BCE/mM creatinine)	Relative Risk	95% C.I
18-38	1.4	0.8 – 2.5
38-51	2.5	1.0 – 6.1
51-67	3.8	1.6 – 9.1
67-188	17.3	2.5 – 118.5

*N = 54, 53, 57, and 55 for each quartile respectively

Use of Osteomark in Patients Diagnosed with Osteoporosis
A study was conducted to establish the safety and efficacy of a new amino-bisphosphonate (alendronate) in the treatment of osteoporosis.[5] The following data support the clinical utility of Osteomark to monitor levels of bone resorption in osteoporotic women treated with antiresorptive therapy (alendronate 10 mg).

- Three months after initiating treatment 80% (71/89) of the subjects had an Osteomark value ≤ 35 nM BCE/mM creatinine and 87% (76/87) had a ≥ 40% decrease from baseline.
- After 1 year of therapy, 90% (77/86) of the subjects had an Osteomark value ≤ 35 nM BCE/mM creatinine and 92% (77/84) of the subjects had a ≥ 40% decrease from baseline.
- Therefore, Osteomark values should be monitored in osteoporotic women treated with antiresorptive therapy to a value of ≤ 35 nM BCE/mM creatinine, or a ≥ 40% decrease from baseline after 3 months.

Use of Osteomark in Patients Diagnosed with Paget's Disease of Bone
A study was conducted to determine the ability of Osteomark to monitor the effect of bisphosphonate therapy on bone resorption in patients diagnosed with Paget's disease of bone. The following data support the clinical utility of Osteomark to monitor the effect of bisphosphonate therapy on bone resorption levels in patients diagnosed with Paget's disease of bone.

Visit	Alendronate	Etidronate	Pamidronate
Month 1*	- 48%	- 39%	- 71%
Month 3**	- 77%	- 58%	- 67%
Month 6***	- 87%	- 72%	- 71%

*N = 22, 17, and 20 respectively
**N = 28, 23, and 20 respectively
***N = 27, 22, and 20 respectively

*Osteomark values should be monitored in Paget's disease of bone patients treated with antiresorptive therapy to a ≥ 30% change or into the normal range.

Table 4.
Percent Responders as Defined by Normalization of Marker (Osteomark 5-65 nM BCE/mM creatinine, total alkaline phosphatase 39-115 IU/L; number of patients with normalized marker value/total number of patients)

Visit*	Osteomark Responders	Total Alkaline Phosphatase Responders
Month 1	19%	2%
Month 3	34%	28%
Month 6	42%	42%

*N = 59, 71, and 69 respectively

- A decrease in total alkaline phosphatase into its normal range is used as an indicator of therapeutic response. Osteomark values also measure the response and provide an earlier assessment than total alkaline phosphatase.

Use of Osteomark to Monitor Estrogen Suppressing Therapy
A clinical trial was conducted to determine the ability of the Osteomark assay to monitor the effect of estrogen suppressing therapy on bone resorption in premenopausal women.[6] The results support the clinical utility of Osteomark to monitor the effect of estrogen suppressing therapy on bone resorption levels.

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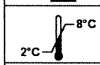
Osteomark NTx Urine Quick Reference

1. Thoroughly read the Assay Procedure before you begin.
2. **Bring kit components and specimens to room temperature.**
3. Dilute the Antibody Conjugate Concentrate into the Antibody Conjugate Diluent, using a 1:101 ratio. Mix gently by inversion only. Use the working strength conjugate solution within one hour of preparation.
4. Pipette 25 µL samples of each Calibrator, Control, and urine specimen into designated microwells.
5. Pipette 200 µL of the working strength conjugate solution into each microwell. Gently swirl to mix. Incubate the plate at room temperature for 90 ± 5 minutes.
6. Prepare Chromogen Reagent/Buffered Substrate solution during the last 10 minutes of incubation. Dilute the Chromogen Reagent into the Buffered Substrate using a 1:101 ratio. Mix gently BY INVERSION ONLY. **Do not vortex, shake vigorously, or mix with a magnetic stir bar. Use the Chromogen/Buffered Substrate within 30 minutes of preparation.**
8. Wash microwells five (5) times with the working strength wash solution at 350µL per well, and blot on absorbent paper towel.
9. Add 200 µL of Chromogen/Buffered Substrate to each microwell, and incubate at room temperature for 15 ± 1 minutes.
10. Add 100 µL of Stopping Reagent to each microwell. Gently swirl the plate to mix.
11. Incubate at room temperature for 5 minutes and read the absorbance of each microwell at 450 nm - 630 nm. Calculate the results using a 4-parameter curve fitting equation.

LOT	Lot No.
IVD	For *In Vitro* Diagnostic Use
	Expiry Date
	Store at 2-8ºC
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