Wampole Laboratories®

OSTEOMARK® NTx Serum

试剂盒说明书

定量检测人血清中的 I 型胶原交联氨基末端肽(NTx)
NTx 是反应骨吸收情况的指标

适用范围

血清 NTx 水平有助于预测骨骼对抗吸收治疗的反应（骨密度），以及监测抗吸收治疗开始后骨吸收的改变。血清 NTx 水平在用于抗吸收治疗前，先是被用于测定绝经后妇女用激素抗吸收治疗和补钙治疗一年后骨密度降低的相关性。

Osteomark NTx Serum 的测量范围是 3.2~40.0nM 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。

交联的 I 型胶原 N 端肽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 α_2N 端肽的定位作用。NTx 分子的产生是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血液和尿液中。

血清中 NTx 作为人类骨吸收的一种标志物，可用 Osteomark NTx Serum 定量测定。血清 NTx 水平的升高即表明骨吸收水平的升高[1, 2, 3, 4]。临床研究表明，骨吸收水平升高是与年龄有关骨损失的首要原因，低骨量常常导致骨质减少，是骨质疏松症的主要原因[5, 6]。据报道，骨质疏松性骨折是老年女性发病率和死亡率升高的主要来源。

在美国的八个临床点对绝经后妇女进行了一次随机试验，受试者随机接受激素替代疗法（HRT）加钙补充（500mg/天），或仅进行钙补充[7]，研究期间收集的血清标本用 Osteomark NTx Serum 法测量。试验结果支持用 NTx 监测抗吸收治疗的效果和测定不用激素治疗一年后骨密度降低的可能性。

在一家地级专业医院对低骨量或诊断为骨质疏松症的绝经后妇女进行了一项随机、双盲临床研究，受试者随机接受安慰剂或 5~10mg alendronate sodium[8]。研究期间收集的血清标本用 Osteomark NTx Serum 法测定，研究结果支持血清中 NTx 用于监测抗吸收治疗的效果和用血清 NTx 值的早期改变预测骨密度对治疗的反应。

测定原理

Osteomark NTx Serum 是一种竞争抑制酶联免疫吸附测定（ELISA/EIA），用于测定人类血清中 NTx。

NTx 表位被吸附到 96 孔微量板中，将稀释后的标本加到微量板孔中，继之加入辣根过氧化物酶标记的单克隆抗体。病人标本中的 NTx 与微量板中的 NTx 表位竞争抗体结合点，经冲洗后，结合的标记抗体量可以通过显色的过氧化物基质比色测定，吸光度可以用分光光度法测量，NTx 的浓度可以通过标准校准曲线计算出来，测定值用 nmol 骨胶原当量/升报告(nMBCE)。

试剂盒组成

所供材料足够 96 人份用

	说明书	1 份
A	抗原包被的 96 孔板 12x8 孔条	1 块
B	标本稀释液	40ml 瓶
C	浓缩抗体结合物	0.4 ml 管
D	抗体结合物稀释液	25 ml 瓶
E	显色剂	0.9 ml 瓶
F	缓冲液	30 ml 瓶
G	终止液	25 ml 瓶
H	30x 浓缩冲洗液	125 ml 瓶
0	0 nMBCE 标准品	20 ml 管
5	5 nMBCE 标准品	0.4 ml 管
10	10 nMBCE 标准品	0.4 ml 管
20	20 nMBCE 标准品	0.4 ml 管
40	40 nMBCE 标准品	0.4 ml 管
I	血清质控品 I	0.4 ml 管
II	血清质控品 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，合成的 NTx 抗原吸附到微孔条中
DILSPE	标本稀释液。1 瓶，用于稀释标准品、质控品、标本的缓冲液，ProClin (0.05%)作为防腐剂。
CONJ	浓缩抗体结合物。1 管，纯化的鼠抗 NTx 单抗，结合有辣根过氧化物酶，ProClin (0.05%)作为防腐剂，浓缩 100 倍。
CONJ DIL	抗体结合物稀释液。1 瓶，用于稀释浓缩抗体结合物的缓冲液，ProClin (0.05%)作为防腐剂。
CHROMOGEN	显色剂。1 管，溶于二甲基亚砜的四甲基联苯胺，浓缩 100 倍。
BUFFER	缓冲基质液。1 瓶，过氧化氢缓冲液，用于稀释显色剂。
SOLN STOP	终止液。1 瓶，1N 硫酸。

WASHBUF	30X

30X 冲洗液。1 瓶，离子清洁液。

CAL	xx nM

标准品：0，5，10，20，40nMBCE。各 1 管，溶于稳定的蛋白稀释液的纯化的 NTx 抗原，ProClin (0.05%)作为防腐剂。

CONTROL	XX

血清质控品 I、II。各 1 管，是已知 NTx 浓度的人类血清，ProClin (0.05%)作为防腐剂。

试剂贮存

试剂不用时必须放 2-8 ºC 保存，用前平衡至室温，不要将试剂暴露于 25 ºC 以上的环境中，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

- 精密的单挡或多挡加样器
- 加样头（测定过程中，每次加入不同样品或试剂时都必须更换新吸头）
- 准备稀释用的微管或类似容器
- 准备工作结合物和显色剂用的一次性塑料容器
- 试剂储存器
- 自动洗板机
- 带有 450nm 和 630nm 滤光片的微量读数仪
- 能用 4 参数对数曲线固定方程计算结果的软件
- 去离子水

标本收集和贮存

通过标准静脉穿刺收集的人类血清可用于 Osteomark NTx Serum 测定，目前，血浆标本尚不能使用。要让血液充分凝固，及时分离血清，对用分离管收集的血清要除去凝胶颗粒。血清标本冷冻状态下（2~8 ºC）可贮存 24 小时，冰冻状态（≤20 ºC）可长期贮存。标本可冻融 3 次。

为了监测治疗效果，应该在治疗开始前或治疗当日收集基准标本，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
- 尤其在监测治疗效果时，不可将 Osteomark NTx Serum 值和 Osteomark NTx Urine 值交替使用。
- 标准品和质控品中含有从人骨组织或血清中提呈的抗原，应当按潜在感染品作适当处理。
- 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，一旦接触皮肤，应立即用水冲洗 15 分钟，如果测入眼中，立即去看医生。TMB 疑有致癌作用。
- 血清样品可能含有传染因子，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 ºC 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。
- 决不要用口吸试剂和样品
- 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。
- 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，不要将干燥剂从锡箔中丢弃，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。

检测步骤

准备步骤：

1. 将标本和试剂平衡至室温（20-25ºC）。将试剂充分混匀，避免泡沫形成。
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，例如用 870ml 去离子水稀释 30ml 浓缩冲洗液）。至少混匀 5 分钟，稀释后的冲洗液在室温可稳定 1 个月。
3. 规化板结构，创立一个板图。建议每份标准品和质控品都做双份，下面是 8 个标本的示例：

	1	2	3
A	0 标准品	40 标准品	3 号标本
B	0 标准品	40 标准品	4 号标本
C	5 标准品	质控品 I	5 号标本
D	5 标准品	质控品 I	6 号标本
E	10 标准品	质控品 II	7 号标本
F	10 标准品	质控品 II	8 号标本
G	20 标准品	1 号标本	9 号标本
H	20 标准品	2 号标本	10 号标本

4. 准备工作强度结合物液。在一个干燥洁净的一次性容器中，用抗体结合物稀释液将浓缩抗体结合物以 1:10 比例稀释，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。容器不可重复使用，按下面图表所示准备试剂

总条数	浓缩结合物(µl)	结合物稀释液(µl)
3 - 4	40	4
5 - 8	80	8
9 - 12	120	12

5. 彻底混匀标准品、质控品和标本。
6. 在微量管或类似容器中，用标本稀释液将标准品、质控品、标本以 1:5 稀释（1 份样品+4 份标本稀释液），每个样品至少需要稀释 200µl（例如 50µl 样品+200µl 稀释液），将稀释样品充分混匀，避免泡沫形成。
7. 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，干燥剂不要丢掉。

标本和抗体的温育：

8. 根据板的布局，各加 100µl 稀释后的标准品、质控品、标本于相应的微量板孔中。建议标准品和质控品都要做双份，加样器要经过校准，每次加样后都要换用一个新吸头。加样完毕，立即转入步骤 9。
9. 向每个加样孔中加入 100µl 工作强度结合物液，盖密封条，在平面上将板轻轻混匀 15-20 秒钟。
10. 在室温（20-25 ºC）温育 90±5 分钟。
11. 在温育的最后 5 分钟准备显色剂和缓冲基质液。在一个一次性使用干净容器中用缓冲基质液将显色剂以 1:101 的比例稀释，颠倒混匀，不要形成涡流、巨烈摇动或使用磁搅拌棒（混匀后溶液应当是无色的，兰色表明试剂被污染，应当弃去），每条测定通常要准备 2ml 溶液(20µl 显色剂＋2ml 缓冲基质液)。
12. 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。最后一遍冲洗后在吸水纸上拍干（过少或过多的冲洗都会造成结果的不准确）。在测定条风干前迅速进入步骤 13。

显色和测量：

13. 每孔中加 200µl 稀释后的显色剂/缓冲基质液，盖一新封条。
14. 室温（20-25 ºC）温育 30±2 分钟，结合有抗体辣根过氧化物酶结合物的孔中会有兰色出现。
15. 温育结束，小心揭去封条。
16. 每孔加 100µl 终止液，兰色孔中颜色将变为黄色，在平面上将板轻轻旋转 15-20 秒钟，将板在室温（20-25 ºC）放置 5 分钟后测吸光度值。
17. 加终止液后 30 分钟内用微量读数仪读取标准品、质控品和标本的吸光度（波长 450nm,参考波长 630nm）。

结果分析

1. Osteomark NTx Serum 值用 nmolBCE/L 表示(nM BCE)。
2. 从标准曲线上取得质控品和样本的浓度值(nM BCE)，最精确的结果可以用四参数对数曲线固定方程式求得（注：某些四参数对数曲线固定方程软件包不接受 0 值标准品，要求输入一个极小的浓度（例如 0.001）代替 0 nM BCE 标准品）。
3. 结果在满足下列条件的情况下是有效的：
   - 0 nM BCE 标准品的吸光度均值≥1.300
   - 标准曲线的范围(0 nM BCE 标准品和 40 nM BCE 标准品的吸光度差距)≥0.900
4. 如果样品作了双份测定，双份测定浓度值的变异系数(%CV)≤20%是可接受的，变异系数>20%的样本应当重测。
5. 病人样本吸光度值<40nMBCE 标准品吸光度值的，应当用 0nMBCE 标准品以 1:2 稀释后重测，如若结果仍低，改用样品稀释液以 1:5 的比例稀释后重测，稀释后样品测定出的浓度值乘以稀释倍数即得原始结果。
6. 制造商已经建立了血清质控范围，建议每个试验室确立自己的质控范围。

局限性

虽然 Osteomark NTx Serum 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、甲状腺机能亢进和佩吉特骨病。用 Osteomark NTx Serum 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。虽然 Osteomark NTx Serum 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 NTx Serum 值不能提供骨吸收率。Osteomark NTx Serum 结果应当结合临床和其他诊断结果综合分析。

干扰物质

对总胆红素、直接胆红素、糖、胆固醇、甘油三脂、总蛋白、白蛋白、血红蛋白等可能干扰 Osteomark NTx Serum 测定的血清成份进行了评估，结果发现，在高于生理水平的情况下均未对测定造成干扰。

预期值

在 5 个地点对停经前健康女性（年龄 25~49 岁，平均年龄 36 岁）的参考范围进行了多重交叉研究，对男性（年龄 31~80 岁，平均年龄为 51 岁）的参考范围在 3 个地点进行了多重交叉研究。

	均值	标准差	范围(均值±2SD)	N
女性	12.6	3.2	6.2 - 19.0	257
男性	14.8	4.7	5.4 - 24.2	176

停经前女性的预期值范围经对数转换后变为 7.7-19.3nM BCE，经对数转换后的男性参考范围为 8.1-24.8nM BCE，此参考范围仅供参考，每个试验室应建立自己的参考范围。为了测定停经后妇女血清中 NTx 的批内变异情况，特地进行了一项研究。受试者连续提供 3 天的血标本用于评估短期变异，连续提供 2 个月的标本用于评估长期变异。短期变异系数均值为 7.3%(n=271)，长期变异系数是 8.7%(n=261)。在对上述男性批内变异的评估中，短期(14 天)批内变异(n=32)是 9.1%，长期(3 月)批内变异(n=27)是 9.5%。

操作特性

测定重复性和精密度

批内变异 是依照 NCCLS 精密度操作规程 EP5-T2，用 4 份 BCE 值遍布标准测量范围的人血清测得的，Osteomark NTx Serum 的批内变异值是 4.6%。

批间变异 是用 BCE 值遍布标准测量范围的 8 份人血清标本测得的，批间变异值是 6.9%。

精密度 通过在 4 家实验室测定血清质控品 I （9.4 nM BCE）和血清质控品 II (30.0 nM BCE)进行评价。

血清质控品 I 的精密度是 13.99%，血清质控品 II 的精密

度是 11.92%。

抗原回收 是通过向 9 个已知 NTx 浓度的血清样品中加入已知量的 NTx 来评价的。回收表示测得值占理论值的百分比，结果表明抗原回收率是 94-105%。

稀释线性 是通过将 5 个高 nMBCE 的血清样品用已知低 nMBCE 值的血清样品做系列稀释来评价的。线性百分数为测得值除以预期值×100，结果表明线性稀释样品的平均回收率是 98%。

临床研究

Osteomark NTx Serum 在用 HRT 治疗的绝经后妇女中的应用

为了检测 Osteomark NTx Serum 对监测 HRT 影响骨吸收的能力，以及补钙治疗与补钙加 HRT 治疗一年后骨密度降底的可能性，进行了一次临床试验，研究结果支持这些论断。图 1a 和图 1b 提供了整个研究过程中，每个治疗组的 Osteomark NTx Serum 值。HRT 开始前，该组 Osteomark NTx Serum 的平均基准值是 15.9nMBCE，显著高于绝经后均值 12.6nMBCE。在 HRT 组，NTx 值在治疗 6 个月后显著降低至于 11.9 nMBCE，平均下降 24.4%（图 2）。钙治疗组，12 个月的研究中均值保持稳定，基准值为 15.4 nMBCE ，12 个月后为 15.8 nMBCE。对 HRT 和补钙组骨损失的风险从 NTx 的基准值进行了比较，在 NTx 基线四分位线的最低值（<12.5NMBCE=，HTR 组和钙组一年后在骨损失的可能性方面无统计学显著差异。如果没用 HRT 治疗，高基准 NTx（>18.1 nMBCE）显示骨密度损失的风险增长了 6 倍。

图 1a. 钙组---整个研究期间 Osteomark NTx Serum 值（图略，请参见原文说明书）

图 1b. HRT 组---整个研究期间 Osteomark NTx Serum 值（图略，请参见原文说明书）

图 2. Osteomark NTx Serum 骨质变化率和变化百分率（图略，请参见原文说明书）

Osteomark NTx Serum 在用双磷酸盐治疗的绝经后妇女中的应用

在美国东北部的一家区级专科医院进行了一项研究，目的是为了检测用双磷酸盐治疗后的 Osteomark NTx Serum 的早期改变是否意味着骨密度的升高 [8]。在这项双盲临床研究中，妇女被随机分组，接受安慰剂或 5~10mg 双磷酸盐。在双磷酸盐治疗组，6 个月后 Osteomark NTx Serum 均值是 11.0 nMBCE，显著低于 16.1 nMBCE 的基准线（图 3a 和 3b）。

图 3a. 安慰剂组---整个研究期间 Osteomark NTx Serum 值（图略，请参见原文说明书）

图 3b. 双磷酸盐组---整个研究期间 Osteomark NTx Serum 值（图略，请参见原文说明书）

图 4，Osteomark NTx Serum 骨密度变化率和变化百分率（图略，请参见原文说明书）

参考文献

略，请参见原文说明书

Osteomark NTx Serum 快速参考指南

1. 先将测定程序通读。
2. 把所有血清标本和试剂放至室温，将试剂彻底混匀。
3. 准备工作强度冲洗液，用去离子水将 30×浓缩洗涤液 1:30 稀释。
4. 安排板结构，给出一个板图。
5. 准备 1:101 稀释的工作强度抗体结合物液，每条约需要 1ml。
6. 在微量管或类似容器中准备 1:5 稀释的标准品、质控品和标本。
7. 根据板图各加 100µl 的标准品、质控品和标本于相应的板孔中。
8. 向各孔中各加入 100µl 工作强度抗体结合物液，盖板，轻轻旋转混匀，在室温温育 90±5 分钟。
9. 在温育的最后 5 分钟，准备 1:101 倍稀释的显色剂/缓冲基质液，每条约需 2ml。
10. 用工作强度冲洗液洗板 5 次，最后 1 次后在吸水纸上拍干。
11. 每孔中加入 200µl 稀释过的显色剂/缓冲基质液，盖板，室温温育 30±2 分钟。
12. 每也中加入 100µl 终止液，轻轻转动混匀。
13. 室温温育 5 分钟，在 450nm-630nm 读取每孔吸光度值，用四参数对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 Serum

Osteomark NTx Serum provides a quantitative measure of cross-linked N-telopeptides of type I collagen (NTx) in human serum as an indicator of bone resorption.

For *in vitro* diagnostic use only

Indications for Use
A Serum NTx level is used to aid in predicting skeletal response (bone mineral density) to antiresorptive therapy and in monitoring bone resorption changes following initiation of antiresorptive therapy. Prior to initiating antiresorptive therapy, a serum NTx level is used to determine the probability for a decrease in bone mineral density (BMD) after one year in postmenopausal women treated with hormonal antiresorptive therapy relative to those treated with calcium supplementation.

The measurement range of Osteomark NTx Serum is 3.2 to 40.0 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 about 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 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 found in urine and serum as a stable end-product of degradation.

Osteomark NTx Serum provides a quantitative measure of NTx in serum as an indicator of human bone resorption. Elevated levels of serum NTx indicate elevated bone resorption.[1,2,3,4] 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.[5,6] Osteoporotic fractures are reported to be the major source of increased morbidity and mortality in older women.

A randomized trial of postmenopausal women was conducted at eight clinical sites across the US. Subjects were randomized to either hormone replacement therapy (HRT) plus calcium supplements (500 mg daily) or calcium supplements alone.[7] Serum samples collected during the study were tested using the Osteomark NTx Serum assay. Results of the testing support the use of Osteomark NTx Serum to monitor the antiresorptive effect of the therapy and to determine the probability for a decrease in BMD after one year if hormone therapy is not initiated.

A randomized, double-blind clinical study was conducted at a regional specialty hospital in postmenopausal women with low bone mass or diagnosed osteoporosis. Subjects were randomized to receive either placebo or 5-10 mg alendronate sodium.[8] Serum samples collected during the study were tested using the Osteomark NTx Serum assay. Results obtained from this study support the utility of Osteomark NTx Serum to monitor the effect of antiresorptive therapy and to predict BMD response to therapy using early changes in the NTx serum value.

Assay Principles
Osteomark NTx Serum is a competitive-inhibition enzyme-linked immunosorbent assay (ELISA/EIA) for quantitative determination of NTx in human serum.

NTx epitope is adsorbed onto a 96-well microplate. Diluted samples are added to the microplate wells, followed by a horseradish peroxidase labeled monoclonal antibody. NTx in the patient sample competes with the NTx epitope in the microplate well for antibody binding sites. Following a wash step, the amount of labeled antibody bound is measured by colorimetric generation of a peroxide substrate. Absorbance is determined spectrophotometrically and NTx concentration calculated using a standard calibration curve. Assay values are reported in nanomoles Bone Collagen Equivalents per liter (nM BCE).

Kit Components
Supplied materials sufficient for 96 wells.

	Instructions for Use	1 booklet
A	Antigen Coated 96-well Plate, 12 1x8-well strips	1 plate
B	Specimen Diluent	40 mL bottle
C	Antibody Conjugate Concentrate	0.4 mL vial
D	Antibody Conjugate Diluent	25 mL bottle
E	Chromogen Reagent	0.9 mL bottle
F	Buffered Substrate	30 mL bottle
G	Stopping Reagent	25 mL bottle
H	30X Wash Concentrate	125 mL bottle
0	0 nM BCE Calibrator	20 mL vial
5	5 nM BCE Calibrator	0.4 mL vial
10	10 nM BCE Calibrator	0.4 mL vial
20	20 nM BCE Calibrator	0.4 mL vial
40	40 nM BCE Calibrator	0.4 mL vial

I	Level I Serum Control	0.4 mL vial
II	Level II Serum Control	0.4 mL vial
	Plate Sealers	1 pad

Reagent Descriptions

PLATE	Antigen Coated 96-well Plate, 12 1x8 well strips. Synthetic NTx antigen adsorbed onto microwell strips.
DILSPE	Specimen Diluent, 1 bottle. Buffered reagent, into which calibrators, controls, and specimens are diluted. ProClin™300 (0.05%) included as a preservative.
CONJ	Antibody Conjugate Concentrate, 1 vial. Purified murine monoclonal antibody directed against NTx and conjugated to horseradish peroxidase. ProClin™300 (0.05%) included as a preservative. Supplied as a 100X concentrate.
CONJ | DIL	Antibody Conjugate Diluent, 1 bottle. Buffered reagent, into which Antibody Conjugate Concentrate is diluted. ProClin™300 (0.05%) included as a preservative.
CHROMOGEN	Chromogen Reagent, 1 vial. 3,3',5,5' - tetramethylbenzidine in dimethylsulfoxide. Supplied as a 100X concentrate.
BUFFER	Buffered Substrate, 1 bottle. Buffered hydrogen peroxide, into which Chromogen Reagent is diluted.
SOLN | STOP	Stopping Reagent, 1 bottle. 1N sulfuric acid.
WASHBUF | 30X	Buffered Substrate, 1 bottle. Buffered hydrogen peroxide, into which Chromogen Reagent is diluted.
CAL | xx nM	Assay Calibrators: 0, 5, 10, 20, 40 nM BCE, 1 vial each. Purified NTx antigen in stabilized protein diluent. ProClin™300 (0.05%) included as a preservative.
CONTROL | xx	Level I and Level II Serum Controls, 1 vial each. Human serum base with known NTx concentration. ProClin™300 (0.10%) included as a preservative.

Storage of Reagents
Reagents must be stored at 2 – 8ºC when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25ºC. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required But Not Supplied
- Precision single and multichannel pipettes.
- Disposable pipette tips. (New pipette tips must be used for each addition of different specimens or reagents during the assay procedure).
- Microtubes or equivalent for preparing dilutions.
- Disposable plastic containers for preparing working conjugate and chromogen solutions.
- Reagent reservoirs.
- Automated microwell washer.
- Microwell or microstrip plate reader with 450nm and 630nm filters.
- Software capable of computing results using a 4-parameter logistic curve-fitting equation.
- Deionized water.

Specimen Collection and Storage
Human serum collected by standard venipuncture technique is used in the Osteomark NTx Serum. The use of plasma samples has not been established. Allow blood to fully clot and remove the serum from the red blood cells promptly. Specimens collected in serum separation tubes should be removed from the gel. Store serum samples refrigerated (2 – 8ºC) for up to 24 hours, or store frozen (-20ºC or below) for longer term storage. Specimens may undergo three freeze/ thaw cycles.

For monitoring therapy, baseline samples should be collected just prior to or on the day of therapy initiation. Subsequent specimens for comparison should be collected at approximately the same time of day as the baseline specimen.

Warnings and precautions
- **For *in vitro* diagnostic use only.**
- Do not interchange Osteomark NTx Serum values with Osteomark NTx Urine values, especially when monitoring therapy.
- The Calibrators and Controls contain processed antigen from human bone tissue or human serum. They should be handled as potentially infectious materials and disposed of appropriately.
- Chromogen Reagent contains 3,3',5,5'- tetramethylbenzidine (TMB) and dimethylsulfoxide (DMSO). DMSO is readily absorbed through the skin. If exposed, flush area with water for 15 minutes. If eyes are exposed, get immediate medical attention. TMB is a suspected carcinogen.
- Serum 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 121ºC. Do not autoclave solutions containing sodium hypochlorite. Do not combine sodium hypochlorite solution with acid.
- Never pipette reagents or clinical specimens by mouth.
- Stopping Reagent contains 1N sulfuric acid. Avoid contact with skin and eyes. If exposed, immediately flush area with water for 15 minutes. If eyes are exposed, get immediate medical attention.
- Do not use reagents beyond their expiration dates.
- Do not mix components from other lots of Osteomark NTx Serum.
- Microwell strips must be stored desiccated. Do not remove the desiccant pillow from the foil pouch, and reseal any unused strips in the pouch with the desiccant pillow.
- Do not re-use 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 kit components to equilibrate to room temperature (20 – 25ºC). Mix all reagents thoroughly. Avoid foaming.
2. Prepare working strength wash solution. Dilute 30X Wash Concentrate 1:30 with deionized water (1 part 30X Wash Concentrate with 29 parts deionized water; example dilution would be 30 mL Wash Concentrate plus 870 mL deionized water) and mix for a minimum of five (5) minutes. The diluted wash solution is stable for one (1) month at room temperature.
3. Plan the plate configuration, and create a plate map. It is recommended that each calibrator and control be run in duplicate. An example for 8 specimens is below:

	1	2	3
A	0 Calibrator	40 Calibrator	Specimen #3
B	0 Calibrator	40 Calibrator	Specimen #4
C	5 Calibrator	Level I Cont.	Specimen #5
D	5 Calibrator	Level I Cont.	Specimen #6
E	10 Calibrator	Level II Cont.	Specimen #7
F	10 Calibrator	Level II cont.	Specimen #8
G	20 Calibrator	Specimen #1	Specimen #9
H	20 Calibrator	Specimen #2	Specimen #10

4. Prepare working strength conjugate solution. Using a clean disposable plastic container, dilute the Antibody Conjugate Concentrate to a 1:101 ratio using Antibody Conjugate Diluent. Mix gently by inversion only. Do not vortex or use a magnetic stir bar. Avoid foaming. Do not reuse the container. Use the following table as a guideline for reagent preparation.

Total Number of Strips	Conjugate Concentrate (µL)	Conjugate Diluent (mL)
3-4	40	4
5-8	80	8
9-12	120	12

Use the diluted conjugate solution within one hour of preparation.

5. Thoroughly mix the Calibrators, Controls and specimens.
6. Prepare 1:5 dilutions of all Calibrators, Controls and specimens with Specimen Diluent in microtubes, or equivalent (1 part sample and 4 parts Specimen Diluent). A minimum volume of 200 µL diluted sample is required for each sample. (e.g. 50 µL sample + 200 µL diluent). Vortex the diluted samples to mix thoroughly, avoid foaming.
7. 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

8. Pipette 100 µL of each diluted Calibrator, Control or sample into the microplate according to the plate configuration. It is recommended that calibrators and controls be run in duplicate. Use a calibrated pipettor and a new pipette tip for each Calibrator, Control, and sample. Immediately proceed to step 9.
9. Using a multichannel pipette, deliver 100 µL of working strength conjugate solution into each microwell. Apply a plate sealer and gently swirl the plate on a flat surface for 15-20 seconds to ensure mixing.
10. Incubate the plate at room temperature (20-25ºC) for 90 ± 5 minutes.
11. Prepare Chromogen Reagent/Buffered Substrate solution during the last 5 minutes of incubation. Dilute Chromogen Reagent into Buffered Substrate using a 1:101 ratio. Use a clean, disposable, plastic container. Do not re-use disposable container. **Mix well by inversion only. Do not vortex, shake vigorously or use a magnetic stir bar to mix.** (This solution should be colorless when mixed. A blue color indicates that the reagent may be contaminated and should be discarded.) As a guideline, prepare 2 mL of solution (20 µL Chromogen Reagent into 2 mL Buffered Substrate) per strip assayed.
12. At the end of the incubation period, carefully remove and discard the plate sealer. Wash microwells five (5) times with the working strength wash solution using an automated plate washer. Use a minimum wash volume of 350 µL per well per wash cycle. Blot on absorbent paper after the final wash. (Too few or too many washes may cause inaccurate results.) Immediately proceed to step 13. Do not allow strips to dry.

Color Development and Measurement

13. Using a multichannel pipettor, add 200 µL diluted Chromogen Reagent/Buffered Substrate to each microwell. Apply a new plate sealer.
14. Incubate at room temperature (20-25ºC) for 30 ± 2 minutes. A blue color will develop in wells containing bound antibody-horseradish peroxidase conjugate.
15. At the end of the incubation, carefully remove and discard the plate sealer.
16. Using the multichannel pipettor, add 100 µL of Stopping Reagent to each well. Wells that have developed a blue color will turn yellow. Swirl the plate gently on a flat surface for 15-20 seconds to ensure mixing. Allow the plate to sit at room temperature (20-25ºC) for 5 minutes before reading absorbance values.
17. Within 30 minutes of adding the Stopping Reagent, read the absorbance of the Calibrators, Controls, and specimens using a microwell plate reader (read at 450 nm with a 630 nm reference filter).

Analysis of Results

1. Osteomark NTx Serum values are expressed in nanomoles BCE/L (nM BCE).
2. Determine the concentration values (nM BCE) of Controls and specimens from the calibration curve. The most accurate results are obtained using a 4-parameter logistic curve fitting equation. [NOTE: Some 4-parameter logistic curve fitting equation software packages do not accept a calibrator value of 0, requiring entry of a nominal concentration (such as 0.001) for the 0 nM BCE calibrator.]
3. Assay results are valid if the following criteria are met:
   - mean absorbance of the 0 nM BCE Calibrator is greater than or equal to 1.300
   - the span of the calibrator curve (absorbance difference between 0 nM BCE Calibrator and the 40 nM BCE Calibrator) is greater than or equal to 0.900.
4. If specimens are run in duplicate, the recommended coefficient of variation (% CV) between concentration value (nM BCE) duplicates is ≤20% CV. Those with > 20% CV should be rerun.
5. Patient specimens giving absorbance values below the 40 nM BCE calibrator should be diluted 1:2 in the 0 nM BCE Calibrator (1 part specimen and 1 part 0 nM BCE Calibrator) before diluting 1:5 in Specimen Diluent, and retested. Calculate final result by multiplying the concentration determined from the diluted sample by a factor of 2.
6. These Serum 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 NTx Serum 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, hyperthyroidism, or Paget's disease of bone. When using Osteomark NTx Serum to monitor therapy, results may be confounded in patients afflicted with other clinical conditions known to affect bone resorption, e.g. metastases to bone. While an Osteomark NTx Serum value provides a measure of the level of bone resorption, a single Osteomark NTx Serum value cannot provide the rate of bone resorption as reported results do not contain a measure of time. Osteomark NTx Serum results should be interpreted in conjunction with clinical findings and other diagnostic results.

Interfering Substances
Various serum components were evaluated for an interfering effect on Osteomark NTx Serum. These components, including total and direct bilirubin, glucose, cholesterol, triglycerides, total protein, albumin and hemoglobin, were tested at levels elevated from physiological norm and did not interfere with assay performance.

Expected Values
A multi-center, cross-sectional study was conducted at five regional sites to determine the reference range for normal premenopausal women (mean age 36 years, range 25-49). The male reference range was determined from a multi-center, cross-sectional study conducted at three regional sites (mean age 51
years, range 31-80).[9]

	Mean*	Std Dev	Range (mean ± 2 Std Dev)	N
Women	12.6	3.2	6.2 – 19.0	257
Men	14.8	4.7	5.4 – 24.2	176

When the expected value range for premenopausal women is log-transformed, the range is 7.7 - 19.3 nM BCE. The log-transformed male range is 8.1 - 24.8 nM BCE. These ranges are provided as guidelines only. Each laboratory should establish their own reference ranges.
A study was conducted to determine the intra-subject variability of serum NTx in postmenopausal women.[10] Subjects provided blood specimens for three consecutive days to assess short-term variability, and for two consecutive months to assess long-term variability. The mean % CV in the short-term specimen set (n=271) was 7.3%. The mean % CV in the long-term specimen set (n=261) was 8.7%.
Intra-subject variability in men was assessed in a subset of the above male reference range study population. The short-term (4 days) intra-subject variability (n=32) was 9.1%, and the long-term (3 months) intra-subject variability (n=27) was 9.5%.

Performance Characteristics
Assay Reproducibility and Precision
Intra-assay variability was determined by testing four human serum specimens with BCE values distributed throughout the calibration range of the assay and following NCCLS Precision Performance Guideline EP5-T2. From these test results the Osteomark NTx Serum intra-assay variability is established as 4.6%.

Inter-assay variability was determined by testing eight human serum specimens with BCE values distributed throughout the calibration range of the assay. From these test results the Osteomark NTx Serum inter-assay variability is established as 6.9%.
Total assay precision was evaluated by testing the Level I Serum Control (9.4 nM BCE) and the Level II Serum Control (30.0 nM BCE) at four clinical laboratories.
The estimate for the total precision % CV for the Level I Serum Control was 13.99%, and for the Level II Serum Control was 11.92%.
Antigen Recovery was evaluated by adding known amounts of NTx to each of nine serum specimens of known NTx concentration. Recovery represented the observed assay value of the "spiked" specimens, calculated as a percent of the expected serum value. Results demonstrated an antigen recovery of 94 – 105% across the assay range.
Dilutional linearity was evaluated by performing serial dilutions of five serum specimens with high nM BCE values into a serum specimen with a known low nM BCE value. Percent linearity was determined as the measured value divided by the expected value multiplied by 100. Results demonstrated an average recovery of linear diluted samples of 98%.

Clinical Studies
Use of Osteomark NTx Serum in Postmenopausal Women Treated with HRT
A clinical trial was conducted to determine the ability of the Osteomark NTx Serum to monitor the effect of HRT on bone resorption and to determine the probability for a decrease in BMD after one year if treated with only calcium supplements relative to those treated with supplements and HRT.7 Results of the study supported these clinical uses. Figures 1a and 1b provide the Osteomark
NTx Serum values throughout the study for each of the treatment groups. Prior to HRT initiation, Osteomark NTx Serum mean baseline value in this group was 15.9 nM BCE, which was significantly higher than the premenopausal mean of 12.6 nM BCE. In the HRT group, NTx values fell significantly after 6 months of therapy to 11.9 nM BCE; a mean 24.4% decrease was observed (Figure 2).
Mean values in the calcium group remained constant throughout the 12 month study; 15.4 nM BCE at baseline and 15.8 nM BCE after 12 months. From the baseline NTx value, the relative risk for loss of BMD was compared between the HRT and calcium-only groups. In the lowest NTx quartile at baseline (<12.5 nM BCE), there was no statistically significant difference in the likelihood of bone loss over 1 year between the HRT and calcium groups. A high baseline NTx (>18.1 nM BCE) indicated a 6 times higher risk of BMD loss if not treated with HRT.

Figure 1a. Calcium Group – Osteomark NTx Serum Values Throughout the Study

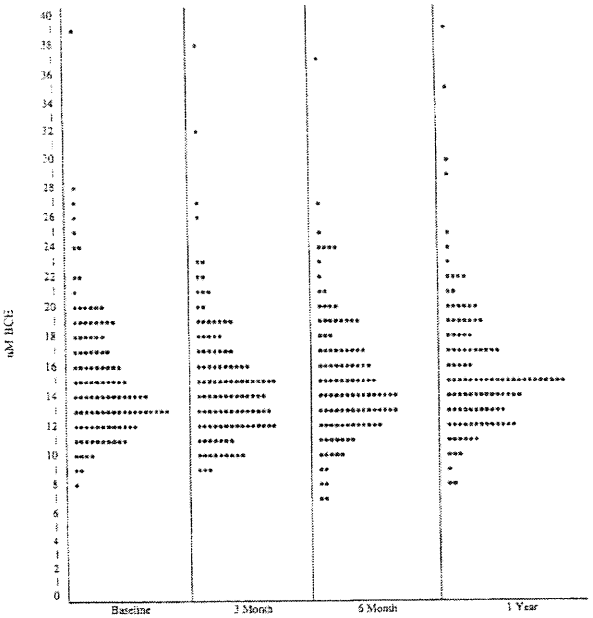


Figure 1b. HRT Group – Osteomark NTx Serum Values Throughout the Study

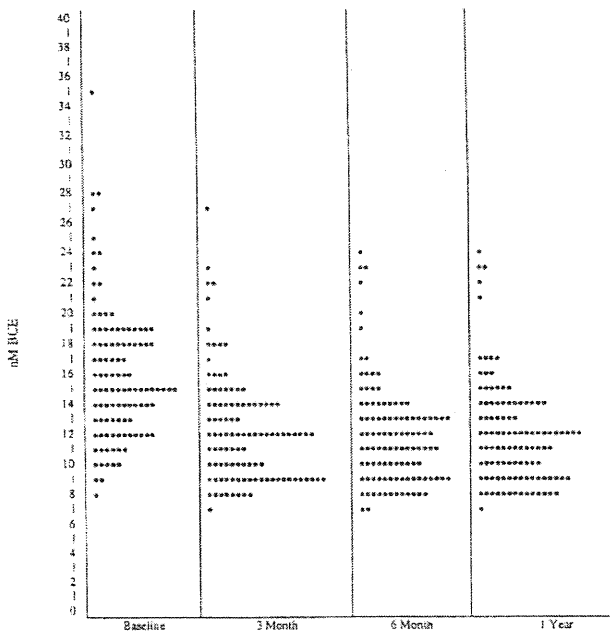
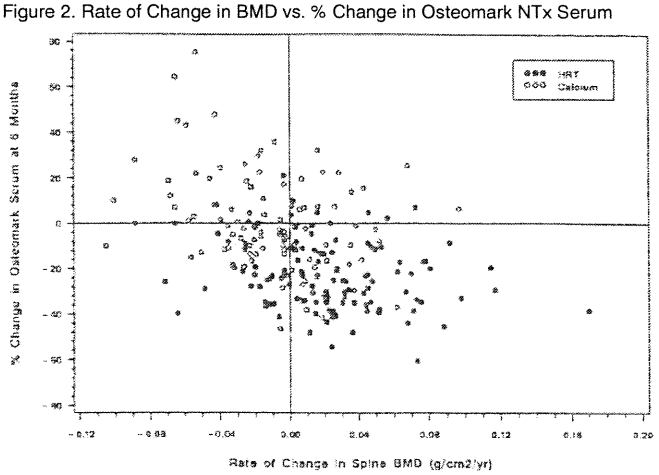


Figure 2. Rate of Change in BMD vs. % Change in Osteomark NTx Serum



Use of Osteomark NTx Serum in Postmenopausal Women Treated with Bisphosphonate Therapy

A study was conducted at a regional specialty hospital in the northeastern United States to determine if early changes in Osteomark NTx Serum following treatment with the bisphosphonate alendronate sodium predicts an increase in BMD.8 In this double-blind clinical study, women were randomized to either placebo or 5-10 mg alendronate sodium. In the alendronate treated group, the mean Osteomark NTx Serum value of 11.0 nM BCE after 6 months of treatment, was significantly lower than the baseline mean of 16.1 nM BCE (Figures 3a and 3b).

Figure 3a. Placebo Group – Osteomark NTx Serum Values Throughout the Study

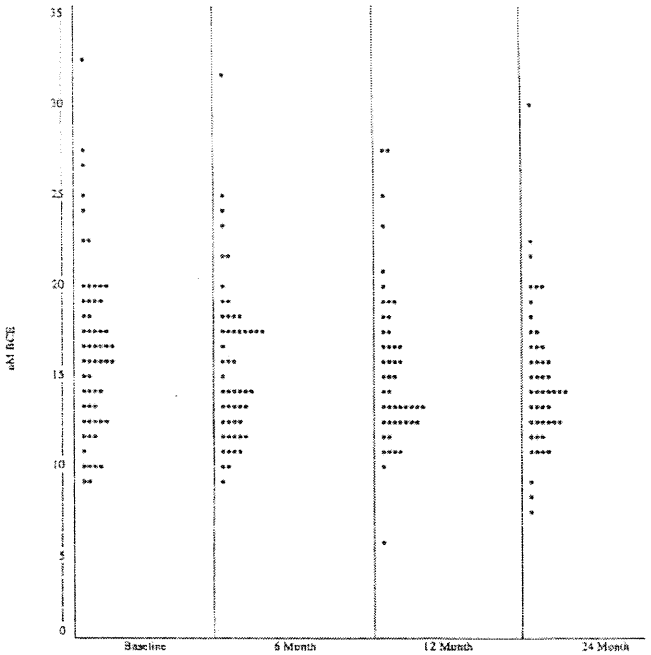
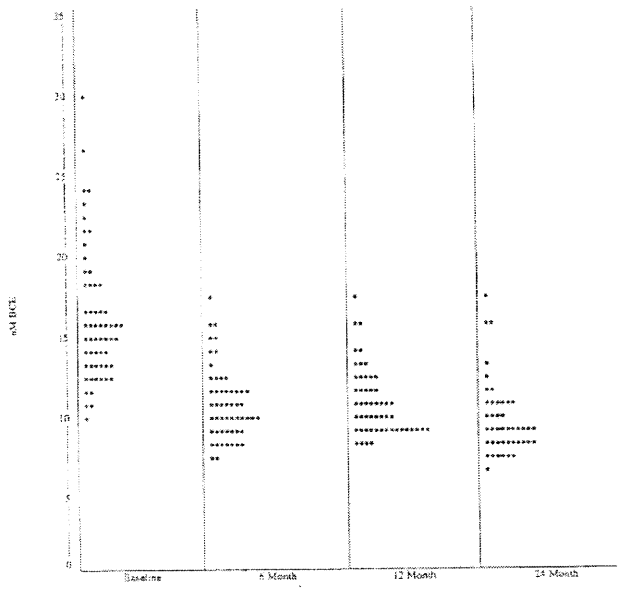
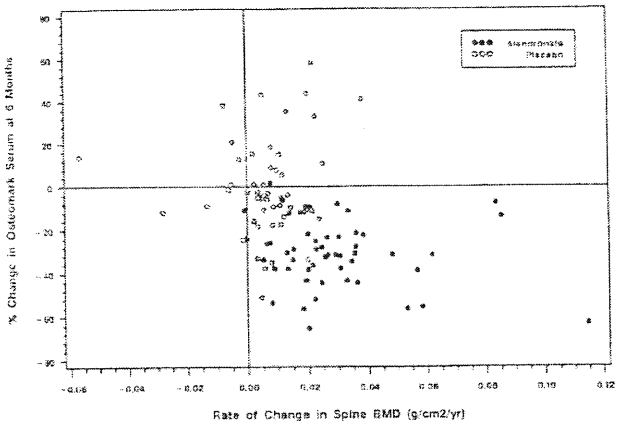


Figure 3b. Alendronate Group – Osteomark NTx Serum Values Throughout the Study



Stratification by tertile of baseline Osteomark NTx Serum value demonstrates that subjects in the highest tertile baseline value (>16.6 nM BCE) had a significantly greater gain in spine BMD than those in the lowest tertile (10.1 – 13.8 nM BCE), p=0.003. Figure 4 provides the rate of change in spine BMD vs. the percent change in Osteomark NTx Serum after 6 months of therapy.

Figure 4. Rate of Change in BMD vs. % Change in Osteomark NTx Serum



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Osteomark NTx Serum Quick Reference Guide

1. Thoroughly read the Assay Procedure before you begin.
2. Allow all specimens and kit components to come to room temperature. Mix all reagents thoroughly.
3. Prepare working strength wash solution. Dilute 30X Wash Concentrate 1:30 with deionized water.
4. Plan the plate configuration and create a plate map.
5. Prepare working strength antibody conjugate solution at a 1:101 dilution. You will need approximately 1 mL per strip.
6. Prepare 1:5 dilutions of all Calibrators, Controls and specimens in Specimen Diluent using microtubes or equivalent.
7. Pipette 100 µL of each diluted Calibrator, Control, and specimen into the microplate according to the plate map.
8. Pipette 100 µL of working strength antibody conjugate solution into each microwell. Cover the plate with a plate sealer, gently swirl to mix and incubate the plate at room temperature for 90 ±5 minutes.
9. Prepare Chromogen Reagent/Buffered Substrate solution at a 1:101 dilution during the last 5 minutes of incubation. You will need approximately 2 mL per strip.
10. Wash microwells five (5) times with working strength wash solution. Blot on absorbent paper after the final wash.
11. Add 200 µL diluted Chromogen Reagent/Buffered Substrate to each microwell. Cover the plate with a plate sealer and incubate at room temperature for 30 ±2 minutes.
12. Add 100 µL of Stopping Reagent to each microwell. Gently swirl the plate to mix.
13. Incubate at room temperature for five (5) minutes and read the absorbance of each microwell at 450 nm - 630 nm. Calculate the results using a 4-parameter logistic curve fitting equation.

LOT	Lot No.
IVD	For *In Vitro* Diagnostic Use
	Expiry Date
2°C 8°C	Store at 2-8ºC
Σ	Contents
REF	Catalog No.

Wampole Laboratories, Inc., Dist.
2 Research Way,
Princeton, NJ 08540 USA

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