

# Biacore 检测抗体与 FcγR II a 相互作用 操作指南



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## 实验目的

利用 Biacore T200 检测抗体与 FcyR II a 结合的亲和力数据 KD。本实验利用 CM5 芯片偶联 anti-his 抗体(或使用 NTA 芯片 + NTA reagent kit, 具体操作参考《Biacore 捕获法检测抗体与 FcRn 相互作用操作指南》),捕获带 his-tag 的 FcyR II a,抗体作为分析物检测亲和力。也可使用 Protein A/G 芯片(Protein A 芯片货号: 29-1275-55, Protein G 芯片货号: 29-1793-15)或 CM5 + human antibody capture kit (货号: 29234600))通过捕获法捕获抗体, FcyR II a 作为流动相进行检测,具体操作可参考《Biacore 捕获法检测抗体与抗原相互作用操作指南》。若 FcyR II a 所带 tag 为 biotin,也可用 SA 芯片(货号: BR-1003-98)或选择 Biotin CAPture Kit, Series S(货号: 28-9202-34)来进行检测,具体操作可参考《Biacore 检测蛋白与核酸相互作用操作指南》。

## 注释

注意事项:实验前请详细阅读该指南,并准备好相应实验用品。该指南仅供类似实验参考,用户须根 据实际样品来源、条件、目的调整各项实验参数。

## 实验使用机型、试剂和耗材

- 本实验所用的机型: Biacore T200, 若为其他机型,请按照对应机型的操作说明进行调整,或咨询 Biacore 产品专家。
- S 系列 CM5 芯片。CM5 芯片货号: 29-1049-88(一片装), BR-1005-30(三片装), 29-1496-03(十 片装), 厂家为 Cytiva。
- 氨基偶联试剂盒(货号: BR-1000-50), 厂家为 Cytiva。
- His 捕获试剂盒(货号: 28-9950-56), 包含 50 μL 1mg/mL anti-his antibody, 1.2 mL Acetate 4.5, 100 mL Glycine 1.5, 厂家为 Cytiva。
- 缓冲液: 10 x HBS-EP+ (货号: BR-1006-69), 厂家为 Cytiva。(也可扫描右侧的二维码选择含上述所有耗材的套餐)
- 去离子水(0.22 µm 膜过滤,若纯水仪已含该滤芯,可无需再次过滤直接使用)。
- 无盖 1.5 mL EP 管(货号: BR-1002-87), 厂家为 Cytiva。
- 带 His-tag 的 FcγR Ⅱ a, 用去离子水溶解稀释至 0.5 µg/mL。

### 实验步骤

#### 仪器准备

#### 开机操作

- 打开 Biacore T200 系统和电脑的电源开关。Biacore T200 的电源开关位于系统背面的右下角。开机 自检通过后(无红灯,温度指示灯闪烁为正常,待系统温度达到设定温度后,面板上的温度指示灯 会停止闪烁),即可操作。
- 打开 Biacore T200 控制软件(Biacore T200 control software), 运行后软件会自动和主机系统建立连接。
- 准备运行缓冲液。量取 50mL 10 x HBS-EP+ buffer、450mL 去离子水(已经 0.22 μm 膜过滤),混匀
   后放入 500 mL 缓冲液瓶。
- 设备开机后,即可使用,无需等待。



#### 缓冲液的放置

- 将已经配制好的缓冲液放在 Biacore T200 系统左侧的托盘上。
- 将缓冲液进液管 A(注意软管上的蓝色标签)插入至缓冲液瓶底部。其余三根进液管(B、C和D) 不要动。
- 将 2L 的废液瓶放置在 Biacore T200 系统右侧的托盘上,并拧上专用的盖子。
- 取 500mL 去离子水装入 500mL 瓶中, 放置在右侧托盘上, 并将标有 water 标签的管子插入瓶中, 用于清洗进样针。

#### 芯片的放置

- 点击工具条中的 🚼 按钮或选择 Tools 菜单中的 Insert Chip 选项,打开芯片舱门。
- 如果已经有芯片在芯片舱内,点击工具条中的 🐨 按钮或选择 Tools 菜单中的 Eject Chip 选项。(若芯片舱中没有芯片,此步直接跳过)



• 如果使用的是新芯片,选择 New Chip。在 Chip Type 的下拉菜单中选择对应的芯片种类(此实验为 CM5 芯片),在 Chip Id 中填入和芯片相关的实验信息,Chip Iot No.中可填入芯片批号(选填)。 如果是已经使用过的芯片,请选择 Reuse Chip,并在 Chip Id 下拉菜单中找到与之相对应的芯片信息。

Insert Chip	
💿 New chip	) O Reuse chip
New chip—	
Chip type:	См5 💌
Chip id:	CM5 21-Feb-08 FrMa
Chip lot no:	(optional)
Help	Dock Chip Cancel

• 手持芯片,有字的一面朝上。按照芯片上的箭头方向,将芯片轻轻推入卡槽,最后合上芯片舱的舱门。



- 点击 Dock Chip 按钮,芯片置入后系统将自动转入待机(Standby)状态。
- 选择 Tools → Prime 命令,点击 Start。缓冲液会以较高的流速冲洗整个内部的流路系统,整个过程 耗时 6-7 分钟。结束后,点击 Close,系统自动转入待机(Standby)状态。注意:当系统开机或更 换缓冲液后,必须运行 Prime 程序。Prime 时缓冲液会冲洗整个流路系统,为下一步的实验做好准备。

#### 放置样品架

• Biacore T200 有三种不同的样品架供用户使用: Reagent Rack 1、Reagent Rack 2(图 A)和 Sample and Reagent Rack1(图 B),见下图。



图 A: Reagent Rack 1&2(左1右2)



图 B: Sample and Reagent Rack1

Reagent Rack 1&2 通常和 96/384 微孔板配合使用,加装在指定的样品架底座上。具体的组装方式参见 下图。



Reagent Rack 1&2 和 96/384 微孔板安装方式

本次实验中使用 Sample and Reagent Rack1。(若待测样品数量多,可选择 Reagent Rack 1 or 2,并根据需要选择是否加 96/384 孔板)。

- 点击工具栏 🎩 按钮,或选择 Tool → Eject Rack,样品舱舱门会自动打开。
- 用手指将样品架底座下方的金属按键向里按(见下图中白色箭头),样品架将会解除锁定并弹出, 然后可以轻轻抽出样品架。



样品架的取出方式

- 按住样品架右侧的黑色按钮, 金属盖会自动弹开。放入相应的样品后, 轻轻合上金属盖。听到"咔哒" 声, 表明金属盖已经处于锁定状态。
- 将样品架沿着卡槽轻轻推入样品舱,听到"咔哒"声,表明样品架已经处于正确位置并锁定。



样品架的放入方式

 点击 Eject Rack Tray 对话框中的 OK,样品架会被自动送入样品舱,舱门也会自动合上。注意:样 品舱舱门打开后会有时间限制,打开 60-90 秒后舱门将自动合上。最后 15 秒时,对话框中的倒数 计时会显示为红色字体并闪烁。此时请不要强行将样品架放入,以免夹到手。可以等待舱门合上后, 重新打开即可。

## 捕获芯片制备

- 1、偶联缓冲液: 1×HBS-EP+。
- 2、Anti-his antibody 偶联(具体操作参考 His capture kit 说明书)

1) 打开 Biacore T200 Control Software, 点击 File 下面的 Open/New wizard template,选择 immobilization。 对话框中,Chip type 选 CM5,在 Flow cells per cycle 选 2。勾选 Flow cell 1.2(如 1.2 已用,可选择 3.4), method 选用 amine 氨基偶联, ligand 输入 anti-his antibody,选用 Specify contact time and flow rate, contact time 为 420s。接着点 Next,选择实验温度,一般默认 25°C。

🚾 Immobilization - Immobilization Setup	5	$\times$
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Flow cells per cycle: 2	~	
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	Ligand: anti-his antibody Dilute ligand	
<ul> <li>Specify contact time and flow rate</li> <li>Blank immobilization</li> </ul>	Contact time: 420 (s) Row rate: 10 (µl/min)	
Flow cell 3,4		
Immobilize flow cell 3,4	Method: Tel Amine	
_	Ligand: Dilute ligand	
$\textcircled{\sc 0}$ Specify contact time and flow rate	Contact time: 420 (s) Flow rate: 10 (µl/min)	
O Blank immobilization		
Help Custom Methods	<back next=""> Close</back>	

2) 在左侧下拉菜单中选用 Sample and Reagent Rack 1,在 Menu 里选 Automatic Positioning 自动排放样 品位置或自行通过鼠标拖拽安排。根据屏幕显示,准备相应的样品,放入的样品体积略大于显示的体 积即可,其中 anti-his antibody用 pH4.5 的醋酸钠稀释至 20 μg/mL。然后,再按要求将不同样品放入 样品架指定位置,如果使用的是带盖的 EP 管,所有盖子必须剪去。盖上样品架金属盖子,将样品架 送回样品舱。

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3) 点击 Next, 弹出 Prepare Run Protocol 对话框,确认各项均符合要求后,点击 start。保存 method 与 result 文件到文件夹(可默认或自行指定,注意本指南中所有要保存的指定文件夹与文件名不可有中 文字符)。系统正式自动运行偶联程序。

4) 偶联结束后,软件自动生成并显示偶联结果。本次实验 fc1 和 fc2 偶联量为 11000RU 左右(具体偶联量视样品实际情况略有差异,在 7000-14000RU 之间即可)。

5) 偶联结束后,即可进入下一步实验,无需等待基线平衡。



## 样品检测过程

 在打开的 Biacore T200 Control Software 里点开 file 中的 Open/New Method, 然后双击打开 Biacore Methods,双击 LMW kinetics。(若只捕获 FcyRIIa,可改用 Wizard,具体的操作参考 Biacore 检测 抗体与 FcyRIIb 相互作用操作指南)

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E Calibration-free concentration	Method Builder	3/18/2013	
E CAP single-cycle kinetics	Method Builder	3/18/2013	
E Fragment Affinity Screen	Method Builder	3/18/2013	
E Fragment Binding Level Screen	Method Builder	3/18/2013	
E Fragment Clean Screen	Method Builder	3/18/2013	
E GST kinetics	Method Builder	3/18/2013	
E Inject and recover	Method Builder	3/18/2013	
E Kinetic Screen	Method Builder	3/18/2013	
E Kinetics heterogeneous analyte	Method Builder	3/18/2013	
E L1 liposome capture	Method Builder	3/18/2013	
LMW kinetics	Method Builder	3/18/2013	
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Help Browse Show	v importable wizard templates	New Op	en Cancel

 在 General Settings 界面,可以修改数据采集频率、检测 Flow cell 数目、分析物浓度单位、样品仓 温度等条件,在此我们将 Concentration unit 改为 μg/mL,若要同时检测不同的 FcγR(3个)与抗体 的互作, Detection 下面可以选 Multi,后面在 setup Run 时选择检测的 Flow path 为 2-1,3-1,4-1。

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 在 Assay Steps 界面, 分别选择 control sample 和 Solvent correction, 点击 Delete 将其删除。在 Cycle Types 界面,选择 Solvent correction, 点击 Delete, 将其删除;选择 Carry-over control 1, 点 击 Remove, 将其删除。

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• 在 Cycle Types 界面,选择 Capture 命令,点击 3 次 Insert 添加捕获命令,选择 Regeneration,点击 Insert 添加再生命令;选择 Sample 1,点击 3 次向下箭头,将样品分析步骤放置在三次不同通道捕获之后、再生之前。

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Etha wash after injection
 Stabilization period:

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在 Cycle Types 界面,点击下方 Capture 1,在其右侧可以修改捕获配体名称、结合时间、流速、流路等进样参数。Capture solution 填写 CD32a, contact time 为 15s (具体时间视样品活性进行调整,以达到捕获量为准。捕获量通常在 35 RU 左右,若样品活性不高,可增加捕获量), Flow rate 为 10 µl/min, Flow path 为 2。同理,点击下方 Capture 2, Capture solution 填写 CD32b, contact time 为 15s (具体时间视样品活性进行调整,以达到捕获量为准。捕获量通常在 45 RU 左右,若样品活性不高,可增加捕获量), Flow rate 为 10 µl/min, Flow path 为 3。同理, Capture solution 填写 CD16b, contact time 为 30s (具体时间视样品活性进行调整,以达到捕获量)。在 Sample 1 一栏中, Contact time 为 60s, Flow rate 为 30 µl/min, Dissociation time 为 60s, 并将 Extra wash 前的 √ 去掉。在 Regeneration 一栏中,再生条件为 Glycine 1.5,再生时间 30s。

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 点击 3 次 Next,进入 Rack Positions 界面,将 Reagent Rack 改为 Sample and Reagent Rack1(若需 要用 96/384 孔板,则选择 Reagent Rack1 或 2,同时在下方 96 well microplate 中选择对应的孔板类型), 点开 Menu 后选 Automatic Positioning, Vial siza 选项选择 Medium,若要合并相同样品,pooling 选 项选择 yes,点击 OK。

ample and Reagent Rack 1		Position	volume (µl)	Content	Туре	Samj Conc (J	ple 1 Jg/ml)	MW (D
14 ( ) ( ) (	RI	D1	236	a	Sample	0		150000
		D2	236	а	Sample	3.125		150000
"O O O11 () (		D3	128	8	Sample	6.25		150000
$\sim$		D4	128	a	Sample	12.5		150000
000		D5	128	a	Sample	25		150000
$\square \cap \cap \bigcup^{\vee} \bigvee$		D6	128	a	Sample	50		150000
		D7	128	a	Sample	100		150000
		D8	560	HBS-EP+	Startup			
		D9	314	CD64	Capture			
♥○○○◎● (	)( ) R1	D10	678	Glycine1.5	Regeneration			
	ヘノー							
$ \begin{array}{c} 5 \\ 6 \\ 4 \\ 6 \\ 3 \\ 2 \\ 6 \\ 6 \\ 8 \\ 8 \\ 8 \\ 8 \\ 8 \\ 8 \\ 8 \\ 8 \\ 8 \\ 8$								
					< Back	Ne	ent >	Close
Image: Second	Postioned by ordering the r	regions. The first Anchor Bottom left	region in the lat. Rack Sample	is positioned first	First Sort By Refer - Ascending	Ne	ext >	Close
Image: Second	P postioned by ordering the r Orientation Column •	regions. The first me Anchor - Bottom left - Sottom left -	region in the lat Rack Sample Sample	s positioned first	First Sort B Ntent - Ascending ntent - Ascending	Ne (	ext >	Close Move Move D
Image: Second	Peet Rack	regions. The first Anchor Bottom left - Bottom left - Bottom left - Bottom left -	region in the latt Rack Sample Reagent Reagent	is positioned first	First Sort By ttent - Ascending ntent - Ascending ntent - Ascending ntent - Ascending	7 	ext >	Close Move Move D

 按照屏幕显示准备相应样品,并按指定位置放置。样品 a 用运行缓冲液 HBS-EP+ 进行倍比稀释。
 点击 Next,对方法进行保存,再对数据路径(可使用系统默认的,也可自行指定,注意所保存的 文件名及指定文件夹名均不能有中文字符)进行保存,仪器便会开始自动运行。

#### 实验结果分析

- 打开 Biacore T200 Evaluation Software,点击 →,找到保存的结果文件。点击左侧 Plot中的 Binding to reference,检查各个点是否趋于一致或小于 binding level 中对应响应值的 20%,再检查 binding level 各个点的响应值是否存在明显的浓度依赖。如是,直接跳到下一步。注:若 Binding to reference 各个点的响应值也存在浓度依赖且大于 binding level 中对应响应值的 20%,即存在非特异 性结合。此时可尝试提高运行缓冲液中盐离子浓度或提高 P20(货号:BR-1000-54)浓度不超过 1%。 若 baseline 中各个点的响应值上飘,可适当延长再生溶液的进样时间。
- 点击上方中间位置的 Kinetics/Affinity,在下拉栏里点击 Surface bound。在 Kinetics/Affinity-Select Curves 界面的 Select evaluation mode 下面选择 Single mode,(若为多组实验结果,并想批量处理, 可选 batch mode;或在 Single mode 模式下,Curve 选择 Fc = 2-1或 3-1或 4-1,分别分析各对互作)。 在跳出的窗口中选择合适的、至少 5 个连续浓度进行拟合。不需要的浓度,可在样品浓度表格中将 此浓度前的对号去掉即可。



 点击右下角 Next,选择右下角 Affinity(当传感图为"时间依赖的动力学特征"时,选 Kinetics), 点击 Next, Model 选择 Steady State Affinity,点击左上角 Fit 进行数据拟合,点击右下角 Finish 完成。
 经拟合,抗体 a 与 FcyR II a 结合的亲和力 KD=1.778×10<sup>-6</sup> M。(对于亲和力拟合,KD 竖线最好落 在样品浓度范围内,并尽量小于最高浓度的一半位置。若 KD 竖线 > 最高浓度,则可提高进样浓度
 梯度,或在上一步选择更高浓度的、至少 5 个连续浓度的样品进行拟合。





• 将鼠标放在图上,点击右键可以直接 copy graph (small, medium, large)用于文章发表,也可以 右键点击 export curve,导出 txt 文本后自行用第三方软件作图。

如有问题,请拨打免费技术热线 请拨 400-810-9118

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