

DuoSet Technical tip

★ 모든 실험 과정은 Datasheet대로 진행 하셔야 합니다 ★

- 1 충분한 warm up 후, kit 사용**
* Warm up 권장시간: 1시간 ~ 1시간 30분 이상
- 2 Capture antibody coating 후, 권장하는 온도에서 overnight incubation**
* 권장하는 overnight 시간: 16 ~ 18시간
- 3 Reconstitution 시, pipetting은 금물!**
* 권장하는 시간 동안 on table,
그 후 vial 벽면에 가루가 남지 않도록 vial을 가볍게 돌리며 녹여 주기.
- 4 Washing 시, well 안의 wash buffer 완벽히 제거**
- 5 실험이 진행되는 동안 Plate가 마르지 않도록 유의**

<CoA보는 법>

SPECIFICATIONS

REAGENT	PART NUMBER	# OF VIALS	AMOUNT PER VIAL	WORKING CONCENTRATION	LOT #
Capture	840125	3	240 µg	4.00 µg/mL	AHZ1923031
Detection	840126	3	15.0 µg	250 ng/mL	WD3023031
Standard	840127	3	100 ng	31.2-2000 pg/mL	P270428
Streptavidin-HRP	893975	3	N/A	40-fold dilution	P364893

PREPARATION & STORAGE

Store unopened kit at 2-8 °C. Do not use past kit expiration date.

REAGENT	PREPARATION	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Capture	Reconstitute with 0.5 mL of PBS	Store at 2-8 °C for up to 8 weeks or aliquot and store at -20 °C to -70 °C in a manual defrost freezer for up to 12 weeks.*
Detection	Reconstitute with 1.0 mL of Reagent Diluent	
Standard	Reconstitute with 0.5 mL of Reagent Diluent	Aliquot and store reconstituted standard at -70 °C for up to 12 weeks.*
Streptavidin-HRP	Dilute with Reagent Diluent	Store undiluted at 2-8 °C for up to 12 weeks. DO NOT FREEZE.*

*Provided this is within the expiration date of the kit.

계산 방법 Ex) Capture antibody Stcok 농도: 240 µg/0.5 mL = 480 µg/mL

Working 농도 : 4 µg/mL → 120배 희석 필요

*Dilution buffer는 Datasheet 확인 (Reconstitution buffer, Dilution buffer는 상이할 수 있음)

DuoSet ELISA 는 **cell culture sup.에서만 validation되어있는 kit**
 다른 sample type으로 실험하실 경우 반드시 사전에 validation test (spike & recovery test) 진행

**재현성 있고 정확한 결과를 위해 추가 구성품을 모아 놓은
 ★Ancillary kit의 사용을 권장 드립니다!**

* kit마다 권장하는 ancillary kit 종류가 다르니, datasheet 확인해 주시기 바랍니다.

DuoSet ELISA Ancillary Reagent kit 1/2/3 (#DY007B, DY008B, DY009B)



* Ancillary kit는 구성품 개별 구매 가능

96-well or 384-well plates
Plate sealers
ELISA plate-coating buffer
TMB ELISA Substrate
Reagent diluent Concentrate 1&3 (DY007B) or Concentrate 2(DY008B) or Concentrate 3 (DY009B)
Wash buffer
PBS (DY007B,DY009B)
Stop solution

그 외 실험에 필요한 시약들

Sample Activation Kit (#DY010)

- 일반적으로 TGF-β 측정에 필요
- Immunoreactivity form을 측정하기 위해 latent TGF-β를 activate 시켜야 함 (Sample 전처리)

Reagent Additive (#DY005)

(Normal Goat Serum, NGS)

- ELISA에서 NGS는 non-specific binding을 막아 background를 낮춰주는 역할
- Detection antibody working concentration으로 희석할 때 datasheet상 안내된 방법대로 넣어서 사용

★ 추가 필요 시약 여부는 반드시 각 kit의 datasheet를 통해 확인해주세요 ★



Troubleshooting your DuoSet® ELISA *continued*

Problem	Possible Cause	Solution
Poor assay to assay reproducibility	Insufficient washing	<ul style="list-style-type: none"> • See washing procedures • If using an automatic plate washer, check that all ports are clean and free of obstructions
	Variations in incubation temperature	<ul style="list-style-type: none"> • Adhere to recommended incubation temperature • Avoid incubating plates in areas where environmental conditions vary
	Variations in protocol	<ul style="list-style-type: none"> • Adhere to the same protocol from run to run
	Plate sealers reused, resulting in presence of residual HRP which will turn TMB blue	<ul style="list-style-type: none"> • Use fresh plate sealer for each step
	Improper calculation of standard curve dilutions	<ul style="list-style-type: none"> • Check calculations, make new standard curve • Use internal controls
	Buffers contaminated	<ul style="list-style-type: none"> • Make fresh buffers
No signal when a signal is expected, but standard curve looks fine	No cytokine in sample or levels below assay range	<ul style="list-style-type: none"> • Use internal controls • Repeat experiment, reconsider experimental parameters
	Sample matrix is masking detection	<ul style="list-style-type: none"> • Dilute samples at least 1:2 in appropriate diluent, or preferably do a series of dilutions to look at recovery
Samples are reading too high, but standard curve looks fine	Samples contain cytokine levels above assay range	<ul style="list-style-type: none"> • Dilute samples and run again
Very low readings across the plate	Incorrect wavelengths	<ul style="list-style-type: none"> • Check filters/reader
	Insufficient development time	<ul style="list-style-type: none"> • Increase development time
	Coated plates are old and have gone bad	<ul style="list-style-type: none"> • Coat new plates
	Capture antibody did not bind to the plate	<ul style="list-style-type: none"> • Use an ELISA plate (not a tissue culture plate) • Dilute in PBS without additional protein
	Buffer containing FCS used to reconstitute antibodies	<ul style="list-style-type: none"> • Requalify your reagents of choice
Green color develops upon addition of stop solution when using streptavidin-HRP	Reagents not mixed well enough in wells	<ul style="list-style-type: none"> • Tap plate
Edge Effects	Uneven temperatures around work surfaces	<ul style="list-style-type: none"> • Avoid incubating plates in areas where environmental conditions vary
		<ul style="list-style-type: none"> • Use plate sealers
Drift	Interrupted assay set-up	<ul style="list-style-type: none"> • Assay set-up should be continuous – have all standards and samples prepared appropriately before commencement of the assay
	Reagents not at room temperature	<ul style="list-style-type: none"> • Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

자세한 학술 상담이 필요하시다면 techserv@woongbee.com 으로 문의주세요.

