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 coating후, 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)

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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 ★

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DuoSet kit에 대한 자세한 정보가 궁금하다면? open

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Troubleshooting your DuoSet® ELISA

Problem	Possible Cause	Solution
High Background	Insufficient washing	• See washing procedure
		• Increase number of washes
		• Add a 30 second soak step in between washes
	Too much streptavidin-HRP or equivalent	• Check dilution, titrate if necessary
	Insufficient blocking	• Check blocking solution calculations
		• Increase blocking time
	BSA impurities	• Use high-quality BSA and consider evaluating a different preparation of BSA
	Incubation times too long	• Reduce incubation times
	Interfering substances in samples or standards	• Run appropriate controls
	Buffers contaminated	• Make fresh buffers
No signal	Reagents added in incorrect order, or incorrectly prepared	• Repeat assay
		• Check calculations and make new buffers, standards, etc.
	Contamination of HRP with azide	• Use fresh reagents
	Not enough antibody used	• Increase concentration
	Standard has gone bad (if there is a signal in the sample wells)	• Check that standard was handled according to directions
		• Use new vial
	Buffer containing FCS used to reconstitute antibodies	• Requalify your reagents of choice
	BSA impurities	• Use high-quality BSA and consider evaluating a different preparation of BSA
	Capture antibody did not bind to plate	• Use an ELISA plate (not a tissue culture plate)
		• Dilute in PBS without additional protein
	Buffers contaminated	• Make fresh buffers
Too much signal—whole plate turned uniformly blue	Insufficient washing/washing step skipped – unbound peroxidase remaining	• See washing procedure
	Substrate Solution mixed too early and turned blue	• Substrate Solution should be mixed and used immediately
	Too much streptavidin-HRP	• Check dilution, titrate if necessary
	Plate sealers or reagent reservoirs reused, resulting in presence of residual HRP. This will turn the TMB blue non-specifically	• Use fresh plate sealer and reagent reservoir for each step
	Buffers contaminated with metals or HRP	• Make fresh buffers
Standard curve achieved but poor discrimination between points (low or flat curve)	Not enough streptavidin-HRP	• Check dilution, titrate if necessary
	Capture antibody did not bind well to plate	• Use an ELISA plate (not a tissue culture plate)
		• Dilute in PBS without additional protein
	Not enough detection antibody	• Check dilution, titrate if necessary
	Plate not developed long enough	• Increase Substrate Solution incubation time
		• Use recommended time
	Incorrect procedure	• Go back to General ELISA Protocol; eliminate modifications, if any
	Improper calculation of standard curve dilutions	• Check calculations, make new standard curve
Poor Duplicates	Insufficient washing	• See washing procedure
		• If using an automatic plate washer, check that all ports are clean and free of obstructions, add a 30 second soak step and rotate plate halfway through the wash
	Uneven plate coating due to procedural error or poor plate quality (can bind unevenly)	• Dilute in PBS without additional protein
		• Check coating and blocking volumes, time and method of reagent addition. Check plate used
		• Use an ELISA plate (not a tissue culture plate)
	Plate sealer reused	• Use a fresh plate sealer for each step
	No plate sealers used	• Use plate sealers
	Buffers contaminated	• Make fresh buffers

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Troubleshooting your DuoSet® ELISA *continued*

Problem	Possible Cause	Solution
Poor assay to assay reproducibility	Insufficient washing	• See washing procedures
		• If using an automatic plate washer, check that all ports are clean and free of obstructions
	Variations in incubation temperature	• Adhere to recommended incubation temperature
		• Avoid incubating plates in areas where environmental conditions vary
	Variations in protocol	• Adhere to the same protocol from run to run
	Plate sealers reused, resulting in presence of residual HRP which will turn TMB blue	• Use fresh plate sealer for each step
	Improper calculation of standard curve dilutions	• Check calculations, make new standard curve
		• Use internal controls
	Buffers contaminated	• Make fresh buffers
No signal when a signal is expected, but standard curve looks fine	No cytokine in sample or levels below assay range	• Use internal controls
		• Repeat experiment, reconsider experimental parameters
	Sample matrix is masking detection	• 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	• Dilute samples and run again
Very low readings across the plate	Incorrect wavelengths	• Check filters/reader
	Insufficient development time	• Increase development time
	Coated plates are old and have gone bad	• Coat new plates
	Capture antibody did not bind to the plate	• Use an ELISA plate (not a tissue culture plate)
		• Dilute in PBS without additional protein
	Buffer containing FCS used to reconstitute antibodies	• Requalify your reagents of choice
Green color develops upon addition of stop solution when using streptavidin-HRP	Reagents not mixed well enough in wells	• Tap plate
Edge Effects	Uneven temperatures around work surfaces	• Avoid incubating plates in areas where environmental conditions vary
		• Use plate sealers
Drift	Interrupted assay set-up	• Assay set-up should be continuous – have all standards and samples prepared appropriately before commencement of the assay
	Reagents not at room temperature	• 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.