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General information on antisera

- Antisera that are not on the list can potentially be produced on request. Please inquire.
- Custom-made conjugates with fluorescent labels or AP can be produced upon request.
- Protocols are available at www.primediagnostics.com.
- The AP-conjugate and coating should be diluted 1000x.
- The coating and alkaline phosphatase (AP) conjugates are suitable for use in DAS-ELISA only.

Storage information and stability

- Coatings and AP-conjugates should preferably be stored at 4 °C. At 4 °C antisera are stable for at least two
 years.
- Controls and IgG should be stored in aliquots at -20 °C. Avoid repeated freezing and thawing. When treated properly these reagents will remain stable for at least two years.
- For long time storage (periods longer than one year) store at -20 °C, prepare aliquots if necessary.
- When storing at -20 °C avoid repeated freezing and thawing, as this can influence the product quality considerably.
- Lyophilized positive control (VPC) contain lyophilized leaf material. The VPC remains stable for 1 year when stored at 4 °C. A prepared VPC can be stored in aliquots of 0.2 mL and is stable for 1 month at -20 °C and at 4 °C for 1 day.
- Lateral flow devices, packed in their original unopened (sealed) envelopes can be stored at room temperature. They will remain stable until expiration date. Lateral flow devices, packed in their original opened envelopes will remain stable for two weeks.
- Luminex reagents should be stored at 4°C, unless indicated different on the box. They will remain stable for one year.

Information concerning the quality of the antisera

- Antisera are biological products and differences in reactivity between batches are apparent. Prime Diagnostics exercises strict quality control and guarantees the reactivity as indicated on the batch quality sheet (if applicable).
- Negative controls are optimized for use in different crops and are not suitable for determination of the selectivity of the assay.
- Positive controls are qualitative and cannot be used for quantification!
- The criteria for reactivity of ELISA reagents for bacterial detection are an OD_{405} reading of the 10 times diluted positive control (end concentration of 10^7 cells/mL) of $OD_{405} > 1.0$ after 30 minutes of substrate incubation.
- The criterion for reactivity of ELISA reagents for virus detection is an OD_{405} reading of the 10 times diluted positive control of at least 0.5 OD after 30 minutes of substrate incubation. The value of the OD_{405} may however vary depending on the ELISA reader used.
- Within our quality control procedure, our antisera are tested for lack of reactivity against a variety of pathogens and/or plant compounds known to occur in combination with the given pathogen. However, within the context of any given test, the possibility of false-positive results with unrelated organisms or plant or matrix substances should always be considered.
- Within our quality control procedure, our antisera are tested for specificity against a wide variety of currently known and recognized pathogenic isolates, strains and patho-types of the subsequent pathogens. However, new pathogenic or non-pathogenic strains and isolates of a given pathogen may emerge over time. Prime Diagnostics cannot guarantee that such a new strain or isolate will be detected with equal efficiency.

Antisera to plant-pathogenic bacteria

- Antisera labeled with different conjugates are available on request. Prices can be supplied upon request.
- For all bacteria non-infectious positive controls are available for use in DAS-ELISA or IIF. These controls have a concentration of 108 cells/mL.
- Negative controls consisting of non-infectious bacteria or plant extract are available on request.
- The dilution of the IgG for IIF is dependent on the assay used and laboratory circumstances. It is recommended to determine the working dilution of the reagents under on-site conditions.
- The IgG is suitable for use in indirect immunofluorescense (IIF) detection.

Virus inocula

• Lyophilized virus particles, dried plant material (to be reconstituted in buffer) or purified virus for inoculating purposes can be obtained on request. The amounts are generally sufficient for small-scale screening experiments.

Orders and invoicing

WhatsApp : +31 317 480 613 e-mail : primediagnostics@wur.nl web shop : shop.wur.nl/primediagnostics web page : www.primediagnostics.com

Contact persons for information

Sales			primediagnostics@wur.nl
Invoicing			primediagnostics.invoices@wur.nl
General information	José van Beckhoven	(+31) 317 480 603	jose.vanbeckhoven@wur.nl
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	Gabriel Lorencini Fiorin	(+31) 317 489 357	gabriel.lorencinifiorin@wur.nl
Luminex	Sander Grapendaal	(+31) 317 484 462	sander.grapendaal@wur.nl
	Henry van Raaij	(+31) 317 480 943	henry.vanraaij@wur.nl
TaqMan	Sander Grapendaal	(+31) 317 484 462	sander.grapendaal@wur.nl
	Henry van Raaij	(+31) 317 480 943	henry.vanraaij@wur.nl

Abbreviations

AP	alkaline phosphatase		
СО	coating		
DAS	double antibody sandwich		
ELISA	enzyme linked immuno sorbent assay		
IIF	indirect immunofluorescence		
IIF	indirect immunofluorescence		
LFD	later flow device		
mL	milliliter		

NC	negative control	
OD ₄₀₅	optical density at 405 nm	
PC	positive control	
pv.	pathovar	
subsp	subspecies	
VPC	lyophilized positive control	
mL	milliliter	

Antisera Virus

The antisera listed below are available for ELISA and Luminex xMAP, LFD format on request. Ordering information and details of these formats can be found in the webshop of Prime Diagnostics: $\frac{\text{shop.wur.nl/primediagnostics}}{\text{shop.wur.nl/primediagnostics}}$.

Pathogen (virus)	Acronym	
Alfalfa mosaic virus	AMV	
Alstroemeria carlavirus	AICV	
Alstroemeria flower-banding virus	AIFBV	
Alstroemeria mosaic virus	AIMV	
Alternanthera mosaic virus	AltMV	
Andean potato latent virus/Andean potato mild mosaic virus	APLV/ APMMV	
Andean potato mottle virus	APMV	
Apple chlorotic leafspot virus	ACLSV	
Apple stem grooving virus	ASGV	
Arabis mosaic virus	ArMV	
Bean common mosaic necrosis virus	BCMNV	
Bean common mosaic virus	BCMV	
Bean yellow mosaic virus	BYMV	
Beet western yellows virus	BWYV	
Bell pepper mottle virus	BePMV	
Calibrachoa mottle virus	CbMV	
Carnation etched ring virus	CERV	
Carnation latent virus	CLV	
Carnation mottle virus	CarMV	
Carnation necrotic fleck virus	CNFV	
Carnation ringspot virus CRS		
Carnation vein mottle virus	CVMV	
Cherry leafroll virus	CLRV	
Chrysanthemum virus B	CVB	
Cucumber green mottle mosaic virus	CGMMV	
Cucumber mosaic virus	CMV	
Cymbidium mosaic virus	CymMV	
Dasheen mosaic virus	DsMV	
Hosta virus X	HVX	
Impatiens necrotic spot virus	INSV	
Iris yellow spot virus	IYSV	
Kalanchoe mosaic virus	KMV	
Leek yellow stripe virus LYSV		
Lettuce big vein associated virus	LBVaV	
Lettuce mosaic virus	LMV	

Pathogen (virus)	Acronym	
Melon necrotic spot virus	MNSV	
Mirafiori lettuce big vein virus	MiLBVV	
Odontoglossum ringspot virus	ORSV	
Onion yellow dwarf virus	OYDV	
Pea early-browning virus	PEBV	
Pea seed-borne mosaic virus	PSbMV	
Pelargonium flower-break virus	PFBV	
Pelargonium line pattern virus	PLPV	
Pepino mosaic virus	PepMV	
Pepper mild mottle virus	PMMoV	
Plum pox virus ('sharka')	PPV	
Potato leafroll virus	PLRV	
Potato virus A	PVA	
Potato virus M	PVM	
Potato virus S	PVS	
Potato virus V	PVV	
Potato virus X	PVX	
Potato virus Y	PVY	
Prune dwarf virus	PDV	
Shallot yellow stripe virus	SYSV	
Squash mosaic virus	SqMV	
Strawberry latent ringspot virus	SLRSV	
Streptocarpus flower-break virus	SFBV	
Tobacco mild green mosaic virus	TMGMV	
Tobacco mosaic virus	TMV	
Tobacco ringspot virus	TRSV	
Tomato aspermy virus	TAV	
Tomato black ring virus	TBRV	
Tomato brown rugose fruit virus	ToBRFV	
Tomato bushy stunt virus	TBSV	
Tomato mosaic virus	ToMV	
Tomato ringspot virus	ToRSV	
Tomato spotted wilt virus TSWV		
Tulip Virus X	TVX	
Zucchini yellow mosaic virus	ZYMV	

Antisera Bacteria and Oomycetes

The antisera listed below are available for IIF, other formats on request. Ordering information and details can be found in the webshop of Prime Diagnostics: $\frac{\text{shop.wur.nl/primediagnostics}}{\text{prime Diagnostics}}$

Pathogen (bacteria)	Acronym
Curtobacterium flaccumfaciens pv. flaccumfaciens	Cff
Curtobacterium flaccumfaciens pv. oortii	Cfo
Clavibacter insidiosis	Ci
Clavibacter michiganensis subsp michiganensis	Cmm
Clavibacter michiganensis subsp sepedonicus	Cms
Dickeya chrysanthemi (Erwinia chrysanthemi)	Ech
Erwinia amylovora	Eam
Pectobacterium atrosepticum	Eca
Pseudomonas chichorii	Рс
Pseudomonas syringae pv. lachrymans	Psl
Pseudomonas syringae pv. mors-prunorum	Psm
Pseudomonas savastanoi pv. phaseolicola	Psph
Pseudomonas syringae pv. pisi	Pspi
Pseudomonas syringae pv. porri	Pspo
Pseudomonas savastanoi pv. savastanoi	Pssa
Pseudomonas syringae pv. syringae (strain specific)	Pssy
Pseudomonas syringae pv. tomato	Pst
Rhodococcus fascians	Rhf
Ralstonia solanacearum	Rsol
Xanthomonas axonopodis pv. dieffenbachiae	Xcd
Xanthomonas phaseoli pv. phaseoli	Xcph
Xanthomonas citri pv. fuscans	Xcphf
Xanthomonas axonopodis pv. begoniae	Xcb
Xanthomonas campestris	Xccam
Xanthomonas axonopodis pv. vesicatoria	Xcv
Xanthomonas axonopodis pv. fragariae	Xf
Xanthomonas hyacinthi	Xch
Xanthomonas hortorum pv. pelargonii	Хср
Xanthomonas arboricola pv. pruni	Xpru

The antiserum listed below is available for LFD, other formats on request. Ordering information and details can be found in the webshop of Prime Diagnostics: shop.wur.nl/primediagnostics

Pathogen (oomycete)	Acronym
Phytophthora	Phyto

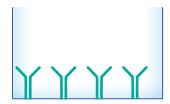
Special terms and conditions of Wageningen Plant Research (WPR) concerning Prime Diagnostics Products

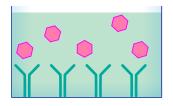
- These "Special terms of delivery" of Wageningen Plant Research (WPR) and the "General Terms and Conditions" of Wageningen University & Research will apply to all offers and all agreements between WPR and client concerning Prime Diagnostics Products. In case of conflict between both terms, these "Special terms and conditions" takes precedence over the "General Terms and Conditions".
- Only webshop and e-mailed orders that have been accepted by WPR will be handled. Orders by phone are not handled.
- Complaints regarding deficiencies of the products delivered by WPR should be deposited within 2 months after the purchase and should be supported by relevant test results obtained with the standard protocol of Prime Diagnostics. In the event that WPR declares a complaint to be founded, WPR will be exclusively obliged to effect performance as agreed upon as yet or to refund the purchase price paid, at WPR's exclusive discretion.
- All agreements between WPR and the clients are governed by Dutch Law only. All disputes shall be handled exclusively by the competent court in The Hague, The Netherlands.
- All products are supplied under the condition that they are for the exclusive use by the client. They may not be sold, integrated into other commercial applications nor handed over to third parties without distribution or supply and license agreement.
- Antisera are biological products and differences in reactivity between batches may occur. Therefore any guaranty
 given by WPR is limited to the relative reactivity in a standard performed DAS-ELISA with a 10 times diluted positive
 control originating from Prime Diagnostics.
- By placing any order with WPR, the client declares that he/she has read and accepts these "Special terms of delivery" as well as the "General Terms and Conditions" of WPR and that he/she has complete knowledge of the current product information.
- Client shall use the material in appropriate containment conditions only for research purposes. In no event WPR shall
 be liable for any use by client of the material or any loss, claim, damage, or liability of whatever kind of nature, which
 may arise from or in connection with this agreement or the use, handling, storage or transport of the material. WPR
 shall be safeguarded by the recipient company against any claim regarding these matters. Any damage or loss to the
 material during transport is at the purchaser's full risk.
- In the event of any liability of WPR, this liability will be limited to the invoice amount for that part of the order to which the liability pertains, on the understanding that this amount shall in no event exceed the amount WPR in such case will receive from its liability insurance.
- Most of the conjugated antibodies are stabilized with bovine serum albumin (BSA). Due to European legislation (EU DIR 1774/2002 and 668/2004) the use of BSA is not allowed in fields of human or veterinary medicine, agriculture, food or cosmetics. By ordering antibodies from WPR client acknowledges that the ordered product, containing BSA, will be used exclusively for research and analytical purposes. If required a form for the written declaration can be obtained on request or downloaded from our website www.primediagnostics.com.
- Most products are usually available from stock and are shipped within two weeks on receipt of the order. However, to ensure timely delivery, orders should be placed 8 weeks prior to the desired delivery date. In the event of force majeure WPR will be entitled to suspend performance of the agreement or to terminate the agreement without recourse to the courts and without any liability towards the client. Force majeure on the part of WPR means any circumstance beyond the control of WPR, for example: strikes, fire, war, damage, transport difficulties, export obstructions, defaults on the part of suppliers and legal bars to manufacture or to supply the products.
- Payment should be made by wire transfer (Euro Base Payment applying code SHA (shared costs)) using IBAN of WPR and BIC/SWIFT of RABOBANK within 30 days after date of the invoice.
- Permits, import charges or client's formalities necessary for import by the client of the ordered products are not the
 responsibility of WPR. It is the responsibility of the client that the client takes care of this before placing an order.
 All necessary permits need to be in the possession of WPR before an order can be shipped and WPR cannot be held
 responsible for any damages or import charges that might occur from import problems. The client has to verify if the
 use of the products is allowed by its government.
- Prices are CPT (Incoterms 2020) in Euros (€), excluding VAT and all orders are charged with € 25.00 for handling.
 Orders exceeding € 5000.00 are free of handling costs.
- Specificity of the antisera is tested against strains known to be pathogenic at the time of the last actual testing. WPR cannot be held responsible for possible false positive or false negative results caused by newly emerging pathogens, developed resistance, pathogen strains or plant or matrix substances.
- The client has to verify the suitability of the products before purchasing the products. When the product information isn't clearly enough the client has to ask WPR for more information.
- For payments the following VAT number of Wageningen Plant Research have to be used:

Domestic: NL811383696B07Foreign: NL806511618B01

• WPR does not accept the responsibility for any direct or indirect damage that might arise from the use of delivered products. WPR shall be safeguarded by the recipient company against any claim regarding the delivery and / or the use of the delivered products.

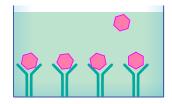
Schematic overview of the DAS-ELISA procedure





Add plant samples to antibody coated microtiterplate wells (top). Sample containing pathogen (left), healthy plant material (right).

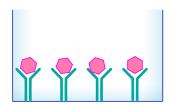




When samples contain the pathogen they will be captured by the antibodies coated to the surface of the microtitre plate (left).

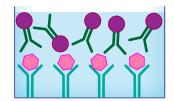
When no pathogens are present





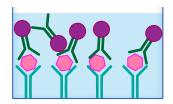
Wash microtitre plate to remove all debris from plant material. The captured pathogens remain captured (left) while in the healhty plant material nothing will remain (right).



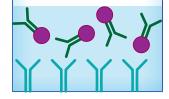


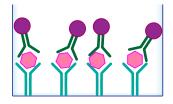
Add alkaline phosphate (AP) conjugated secondary antibodies.



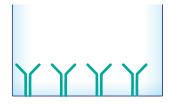


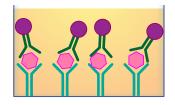
When samples contain the pathogen AP-conjugated antibodies will bind to the captured pathogens (left). When no pathogens were captured the AP-conjugated secondary





Wash microtitre plate. The AP-conjugated secondary antibodies remain captured (left) while in the healhty plant material nothing was captured and will remain (right).



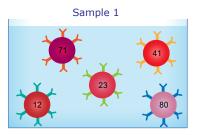


Examine result after adding substrate and incubating for 30 minutes.

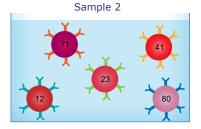


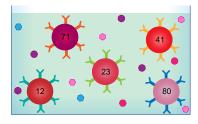
Schematic overview of the Luminex xMAP procedure

The bead mixtures used contain hundreds of beads of different bead addresses. Each bead address is conjugated to one (1) specific antibody for one (1) specific plant pathogenic organisms. This makes it possible to test for multiple pathogens simultaneousy, each bead address - plant pathogen combination is specifid for the pathogen it was designed for.

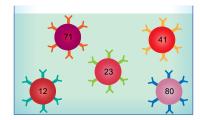


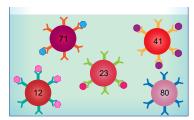
Add conjugated bead antibody mixture (hundreds of beads of the five bead addresses 12, 23, 41, 71 and 80, each conjugated with a specific antibody) to the microtiterplate wells.



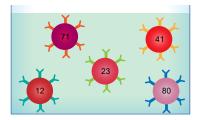


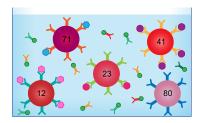
Add plant samples to microtiterplate wells, mix and incubate.



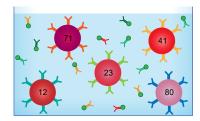


During incubation the plant pathogens will be captured by the specific antibodies conjugated to their bead address (left). If no pathogens are present nothing will be captured (right).



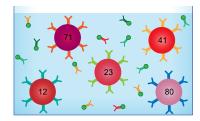


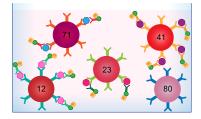
After a wash step a mixture of biotinylated secondary antibodies is added and incubated.



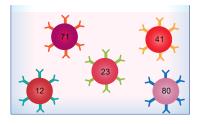


During this incubation the biotinylated secondary antibodies will bind to the plant pathogens (left). If no pathogens are present no biotinylated secondary antibodies will bind (right).





After a wash step all antibodies, that didn't bind are removed and a dye (SA-PE) is added. The SA-PE will bind to the biotine and after a short incubation the samples can be measured. The higher the signal the more of the detected plant pathogen present in the sample.



Sample	Plant pathogen				
Sumple	1 2 3 4 5				
	(bead 12)	(bead 23)	(bead 41)	(bead 71)	(bead 80)
1	2500	200	1356	230	28
2	27	30	29	33	31
n	X	X	X	X	X

Schematic overview of the TaqMan procedure

After DNA isolation specific primers and probe are added to the sample. The probe consists of quencer and a fluorescent molecule. When fluorophore and quencher are in proximity, quenching inhibits any fluorescence signals.

When the target is present the primers and probe will bind to the DNA (left) at a specified temperature. If the target isn't present the primers and/or probe wil not bind (right).



Next, the temperature is decreased and polymerase will elongate the primers.



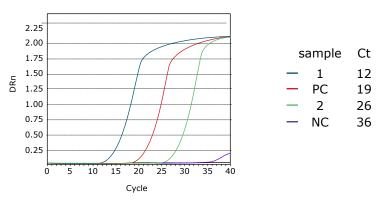
During elongation the polymerase will displace and cleave the probe, releasing a fluorescent molecule which will be measured (left). The fluorescence detected in the quantitative PCR is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. If the probe did not bind, it is not cleaved and no fluorescent molecule will be released.



After increasing the temperature the DNA denaturates and two single DNA strands are formed and the whole proces of binding and elongation can start over again. Now with the doubled amount of targets.



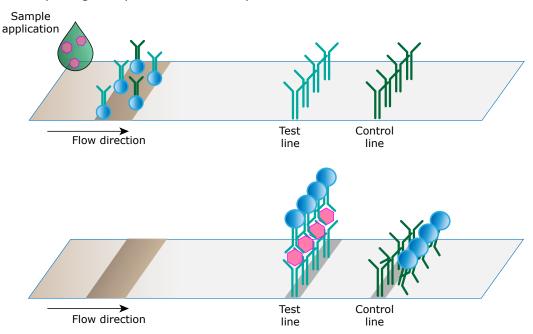
The above steps can be repeated up to 40 times, and each repetition more fluorescent molecules will be released. In this way the signal is amplified exponentially. When plotted in a graph it will look as following:



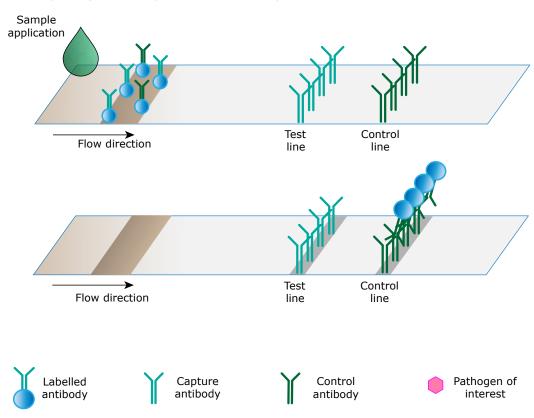
Schematic overview of the Lateral Flow Device procedure

A sample of plant sap is applied to the strip. The control line confirms the test worked properly. A visible test line at the test line region indicates the presence of the pathogen. If the pathogen is not present in your sample, no test line will appear, indicating a negative result.

When pathogen is present in the sample



When pathogen is not present in the sample



Comparison between ELISA and Luminex xMAP

ELISA	Luminex xMAP		
Workflow			
1. Coat microtitre plate	1. Sample extraction		
2. Sample extraction	2. Add bead mixture to the wells of microtitre plate		
3. Transfer samples to the wells of microtitre plate	3. Transfer samples to the wells of microtitre plate		
4. Incubate and wash	4. Incubate and wash		
5. Add secondary antibody-AP conjugate	5. Add secondary antibody mixture		
6. Incubate and wash	6. Incubate and wash		
7. Add reporter	7. Add streptavidin-R-phycoerythrin		
8. Incubate and analyse	8. Incubate, wash and analyse		
 Multiplex			
No, multiplex not possible	Yes, up to 50 targets simultaneous detectable		
S	Speed		
Depending on incubation times 16 to 24 hours	Finished in less than 4 hours		
Consumables and labour input	Consumables and labour input		
For each new target extra amounts of consumables and labour needed	Consumables and labour input not influenced by number of targets (pathogens)		
FI	exible		
No, coated or pre-coated microtitre plates needed	Yes, on demand the tests can be performed		
Generaly, whole plate has to be used	Depending on sample numbers partial plates can be used		
Evaporation of samples in the wells at the edges of the plate	All wells of microtitre plate can be used		
Reliability			
Duplos needed for reliable results	No duplos needed, for each targets more than 50 repetitions are measured		

Accessing webshop and creating an account

To access the web site of Prime Diagnostics scan the QR code:

Or <u>click here</u> to go directly to our webshop



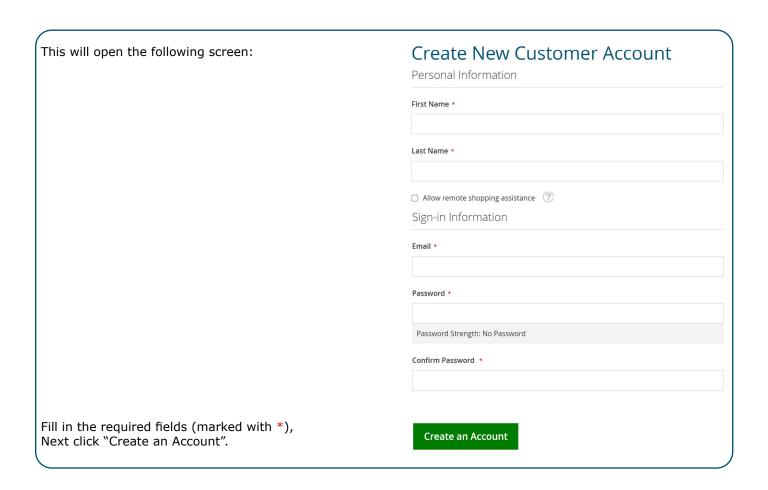
If you already created an account: login

If you are a first time user: create an account. You can follow the steps in the guide shown below.

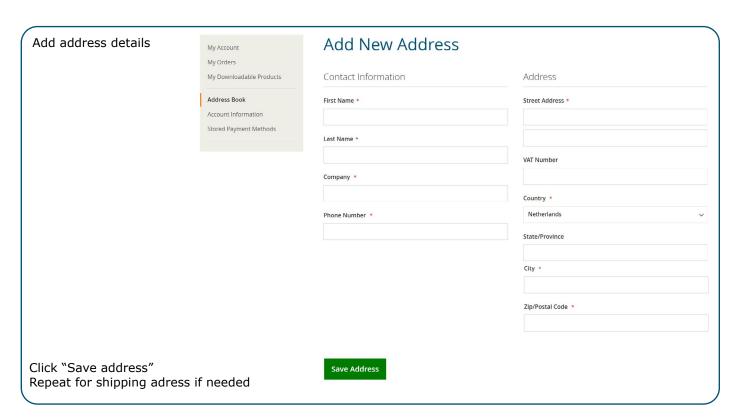
Select "Create an Account" in the upper right corner of the webshop

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Once this is completed you will be informed by e-mail that you have created your account.

Before the first order can be made, and to confirm that the account was made by you, the account needs to be activated. Mail a reply to Prime Diagnostics (primediagnostics@wur.nl) to confirm that your account needs to be activated.

After activation you have full access to the webshop of Prime Diagnostics.

primediagnostics

Prime Diagnostics provides high quality reagents for the detection of plant pathogens in various formats:

- ELISA
- Immunofluorescence
- Inocula
- Lateral Flow Devices
- Luminex xMAP and xTAG
- TaqMan

Prime Support:

From starters to experienced users in the field of plant diagnostics we offer 'state of the art' training with emphasis on improvement of the overall quality of the existing laboratory.

- Companies who want to start up plant diagnostic laboratory activities, Prime Support, a service from Prime Diagnostics can assists in the first steps to start-up plant laboratory activities.
- To existing plant health diagnostics laboratory operations Prime Support offers training to improve skills in plant diagnostics.

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email: primediagnostics@wur.nl

web site : www.primediagnostics.com

web shop: shop.wur.nl/primediagnostics