TEST FORM	INNO – BIA AlzBio

DATE : .....
OPERATOR : .....
PURPOSE : ....
....

1. TEST PROTOCOL

STEP	PROTOCOL	SPECIFICATIONS	RESULTS
DAY-1 PREPARATION	Control on calibration status for instruments	Calibration conform	OK / NOK
	Control for calibration status of pipettes.	Calibration conform	OK / NOK
	Bring all components (Filter plate, DIL, coated beads, Conjugate 1) to room temperature (18-30°C) before start of the assay.	Min: 30 min. Max: 120 min.	START: ..... END: .....
	Place WASH SOLN 25x bottle in warm-water bath at ± 37°C for 30 min. Note: Salt crystals may be formed in the concentrated wash solution after storage at 2 - 8°C.	Min: 30 min. Max: 120 min.	START: ..... END: .....
	Thaw one series of calibrators (Six vials), Control A, Control B, QC Samples, and test samples, on the bench for at least 15 min. before the start of the assay.	Min: 15 min. Max: 60 min.	START: ..... END: .....
	Preparations 1. **Wash solution** : dilute 1/25 with distilled or deionized water 2. **Bead** work solution Note: Cover with aluminium foil or store in the dark - vortex the vial with coated beads 100x (10 sec) - sonicate the vial for 3 min. - vortex the vial with coated beads 100x (10 sec)	Preparation documented (see Dilutions) Preparation documented (see Dilutions)	OK / NOK OK / NOK

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	- dilute 1/100 with DIL *in polypropylene tubes* - vortex and cover the tube with aluminium foil to protect from daylight. 3. **Conjugate 1 Working solution**: - vortex CONJ 1 (stock-solution) (10 sec) - dilute 1/100 with DIL - vortex Working solution (10 sec)	Preparation documented (see Dilutions)	OK / NOK
DAY-1 **ASSAY**	**Filter Plate** - dispense 225 µL of diluted WASH SOLN into each test well. - aspirate WASH SOLN with the Multiscreen Vacuum Manifold until all fluid has been removed and proceed immediately to the next step. *Note: Directions for washing* - *Center the filter plate on the vacuum manifold by putting well A1 on the top left corner.* - *Look at the waste volume. If the waste is full, remove the waste first.* - *Use plate sealers to cover unused wells.* - *Push the plate against the instrument and press the ON (or open position) button.* - *The liquid must be aspirated completely from all wells.* - *After aspiration, press the OFF button(or close position) and fill the wells with 225 µl of diluted wash solution.* - *Remove the liquid from the wells by pressing the ON button again, and pushing the plate down on the vacuum manifold. -Perform this step 3 times, consistently, and without time intervals between washes.* *Incomplete washing will adversely affect the test outcome.* *Microbial contamination of wash solution can cause extensive problems.*	225 µL/well	OK / NOK
	Bead working solution - vortex the prepared bead mix and transfer	100 µL/well	OK / NOK

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	prepared bead mix to the wells of the filter plate - aspirate within a period of 5 min and proceed immediately to the next step *Note: Protect from daylight until next step. Beads should always be covered by fluid* Vortex the Conjugate 1 working solution. Add **Conjugate 1** working solution into each well Vortex (at least 10 sec) the thawed calibrators, Control A, Control B, QC- Samples, and Test Samples. Add **Calibrators**, **Controls**, **Samples**, or **DIL** to the filter plate. Cover plate with aluminium foil or store in the dark.	25 µL/well 75 µL/well	OK / NOK OK / NOK
	Start overnight incubation at room temperature on an orbital plate shaker.	Temp (°C) Min: 18 ; Max: 30 Time (hrs): Min: 14; Max: 18	TEMP – START: …… TEMP – END : …… TIME – START: …… TIME – END : …… OK/NOK
DAY-2 **ASSAY**	Bring all components (including prepared WASH SOLN, DIL, READ SOLN, DETECT CONJ) to room temperature (18-30°C).	Min: 30 min. Max: 120 min.	TIME – START: …… TIME – END : ……
	Preparations **1. DETECT CONJ** - vortex DETECT CONJ 100x - dilute 1/100 with DIL - vortex DETECT CONJ working solution - cover with aluminium foil to protect from daylight.	Preparation documented (see Dilutions)	OK/NOK
	Wash Procedure - unwrap filter plate and aspirate bead-sample	225 µL/well (3 times)	

	mix using the vacuum manifold until all fluid has been removed. - dispense diluted WASH SOLN to each well and aspirate using vacuum manifold. Stop aspiration when all fluid has been removed.		OK/NOK
	START-UP procedure of Luminex instrument, including calibration		
	Conjugate incubation Dispense Detection Conjugate solution into each test well. Cover plate with aluminium foil or store in the dark. Incubate for 60 min at room temperature on an orbital shaker.	100 µL/well Time (Min): Min: 58; Max: 62 Temp (°C): Min: **; Max:**	TIME – START: …… TIME – END : …… TEMP : ……°C
	Wash Procedure - unwrap filter plate and aspirate bead-sample mix using the vacuum manifold until all fluid has been removed. - dispense diluted WASH SOLN to each well and aspirate using vacuum manifold. Stop aspiration when all fluid has been removed	225 µl/well (3 times)	OK/NOK
	Reading solution - dispense READ SOLN into each well - cover plate immediately with aluminium foil or store in the dark. - place filter on an orbital shaker for at least 2 min. - unwrap the filter plate and place it in the XY platform of the Luminex100 IS to start reading.	100 µl/well Read max. one hr after stop of the reaction	Time (min) after ASSAY-END: …….. TIME – START: …… TIME – END : ……

2. DILUTIONS

DILUTION PROTOCOL		
Total number of wells needed: …….		
Buffer	***Volume requirements***	***Preparation Protocol***
WASH SOLN	20 mL (25X) + 480 mL (for one plate)	WASH SOLN 25x : …….. mL Water : …….. mL
BEAD Working solution (1/100)	Number of strips x 800 µL + 1000 µL	BEADS : ……..µL DIL : ……..µL
Conjugate 1 working solution (1/100)	Number of strips x 200 µL + 1000 µL	Conjugate 1 : ……..µL DIL : ……..µL
DETECT CONJ working solution (1/100)	Number of strips x 800 µL + 3000 µL	DETECT CONJ : ……..µL DIL : ……..µL

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3. INSTRUMENTS

INSTRUMENT	Reference number (if available)	Comments
Water Batch		
Incubator 25°C		
Sonication Bath	Type: Reference:	
Vacuum Manifold Holder	Type: Reference:	Pressure settings:
Orbital shaker	Type: Reference:	Rotation speed:
Luminex	Type : Supplier : Software version :	Last calibration: System settings: - Counted beads : ..... - Sheath flow (µL/sec) : ..... - Volume taken up : ..... - Gate settings : ..... Reading – Start : ..... Reading – End : .....

4. REAGENTS

TEST: INNO-BIA AlzBio3		
COMPONENT	**CODE**	**EXP. DATE**
FILTER PLATE		
COATED BEADS		
CONJ 1 100x		
DETECT CONJ 100x		
DIL		
READ SOLN		
WASH SOLN		
STAND 1		
STAND 2		
STAND 3		
STAND 4		
STAND 5		
STAND 6		
CONTROL A		
CONTROL B		

COMMENTS: If other products are used than mentioned in the kit insert, specify.

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SAMPLES:

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SPECIFICATIONS:

o See Addendum ****

RESULTS:

o Cfr. Addendum ***

o Store *.csv file.

CONCLUSION :

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o PERFORMANCE OF THE TEST OK/NOK

PERFORMER :..... DATE :.....

CONTROL :..... DATE :.....

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