



User's Manual

DGP-IgA ELISA

Enzyme Immunoassay for the determination of IgA antibodies against deamidated Gliadin-specific peptides (DGP) in human serum

REF AE29020

 96

RUO

For Research Use Only – Not for Use in Diagnostic Procedures

1 INTENDED USE

This DGP-IgA ELISA is a solid phase enzyme immunoassay employing synthetic, deamidated gliadin-derived peptides for the quantitative and qualitative detection of IgA antibodies against deamidated Gliadin-specific peptides (DGP) in human serum.

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2 INTRODUCTION

Gluten-sensitive enteropathy or celiac disease is characterized by atrophy of the small intestinal villi leading to a so-called flat mucosa. It is caused by a pathological intolerance to Gliadin, the alcohol-soluble fraction of gluten in wheat, rye and barley. As celiac disease is caused by the uptake of gluten, consequently a gluten-free diet cures the disease completely and thus has to be maintained for life-time. Renewed consumption of Gliadin leads to a return of the symptoms. The disease is HLA-associated (>95% of individuals have DQ2 enREFd by DQA1*0501 and DQB1*0201) and manifests at any age with a peak onset in early childhood, even in neonatals. The incidence rates range from 1 in 4000 to 1 in 300 in European countries.

3 PRINCIPLE OF THE TEST

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. The antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the sample.

4 REAGENTS

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 X 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	1 x 1.5ml	Blue	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgA	1 x 15ml	Red	Red	Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H ₂ O ₂)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

5 STORAGE CONDITIONS

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

6 WARNINGS AND PRECAUTIONS

1. **CAUTION:** This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.²⁵
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. For research use only, not for use in diagnostic procedures.

7 INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

8 SAMPLE COLLECTION AND PREPARATION

1. The use of SERUM samples is required for this test.
2. Samples should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells within 60 minutes after collection.
3. Samples which cannot be assayed within 24 hours of collection should be frozen at -20°C or lower, and will be stable for up to six months.
4. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).
5. Samples should not be repeatedly frozen and thawed prior to testing. DO NOT store in "frost free" freezers, which may cause occasional thawing. Samples which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

9 PROCEDURAL NOTES

1. Pipetting Recommendations (single and multi-channel). Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

10 PREPARATION OF REAGENTS AND SAMPLES

All reagents should be brought to room temperature (18°C - 25°C) before use.

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

To avoid mistakes we suggest to mark the cap of the different calibrators.

Samples:

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 μl sample buffer (1x) + 10 μl serum. Mix well !

Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 μl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly ($2-8^{\circ}\text{C}/35-46^{\circ}\text{F}$).

11 ASSAY PROCEDURE

We suggest pipetting calibrators, controls and samples as follows:



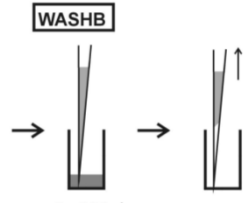
For <i>QUANTITATIVE</i> interpretation					For <i>QUALITATIVE</i> interpretation				
	1	2	3	4...		1	2	3	4...
A	Cal A	Cal E	P1		A	NC	P2		
B	Cal A	Cal E	P1		B	NC	P2		
C	Cal B	Cal F	P2		C	CC	P3		
D	Cal B	Cal F	P2		D	CC	P3		
E	Cal C	PC	P3		E	PC	...		
F	Cal C	PC	P3		F	PC	...		
G	Cal D	NC	...		G	P1	...		
H	Cal D	NC	...		H	P1	...		

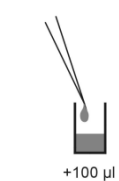
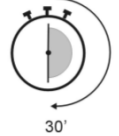
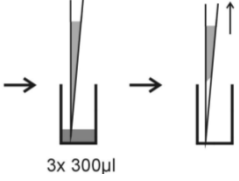
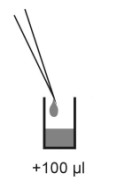

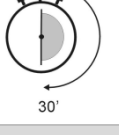
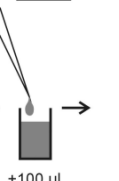


Cal.A: calibrator A
 Cal.B: calibrator B
 Cal.C: calibrator C

Cal.D: calibrator D
 Cal.E: calibrator E
 Cal.F: calibrator F

PC: positive control
 NC: negative control
 CC: cut-off calibrator

P1: sample 1
 P2: sample 2
 P3: sample 3

Step	Description
1.	Ensure preparations from step 10 above have been carried out prior to pipetting.
2.	Use the following steps in accordance with quantitative/ qualitative interpretation results desired:
CONTROLS & SAMPLES	
3.	 <p>Pipette into the designated wells as described above, 100 µl of either:</p> <ol style="list-style-type: none"> Calibrators (CAL.A to CAL.F) for <i>QUANTITATIVE</i> or Cut-off Calibrator (CC) for <i>QUALITATIVE</i> interp. <p>and 100 µl of each of the following:</p> <ul style="list-style-type: none"> Negative control (NC) and Positive control (PC), and Sample diluted serum (P1, P2...)
4.	 <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p>
5.	 <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p>

CONJUGATE	
6.	<div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px; margin-right: 10px;">CONJ</div>  </div> <p>Pipette 100 µl conjugate into each well.</p>
7.	 <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p>
8.	<div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px; margin-right: 10px;">WASHB</div>  </div> <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p>
SUBSTRATE	
9.	<div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px; margin-right: 10px;">SUB</div>  </div> <p>Pipette 100 µl TMB substrate into each well.</p>
10.	<div style="display: flex; align-items: center;">   </div> <p>Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.</p>
STOP	
11.	<div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px; margin-right: 10px;">STOP</div>  </div> <p>Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.</p>
12.	 <p>Incubate 5 minutes minimum.</p>
13.	<p>Agitate plate carefully for 5 sec.</p>
14.	<div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px; margin-right: 10px;"> OD₄₅₀ - OD₆₂₀  </div> <p>Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.</p> </div>

12 INTERPRETATION

For **quantitative interpretation** establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

Normal Range	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

Example of a standard curve

Do not use this example for interpreting your results!

Calibrators	OD 450/620 nm	CV % (Variation)
0 U/ml	0.040	0.0
3 U/ml	0.126	1.5
10 U/ml	0.287	2.7
30 U/ml	0.513	4.0
100 U/ml	1.086	4.5
300 U/ml	1.974	5.6

Example of calculation

Sample	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	1.007/1.011	1.009	98.4
P 02	0.533/0.523	0.528	38.2

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and population according to their own established procedures.

In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated.

The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the manufacturer or the supplier of the test kit.

For **qualitative interpretation** read the optical density of the cut-off calibrator and the samples. Compare samples OD with the OD of the cut-off calibrator. For qualitative interpretation we recommend to consider sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

Negative:		OD sample	<	0.8 x OD cut-off		
Equivocal:	0.8 x	OD cut-off	≤	OD sample	≤	1.2 x OD cut-off
Positive:		OD sample	>	1.2 x OD cut-off		

13 TECHNICAL DATA

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	90 minutes at 20-32°C/68-89.6°F
Calibration range:	0-300 U/ml
Analytical sensitivity:	1.0 U/ml
Reportable range:	1.74 – 300 U/ml
Storage:	at 2-8°C/35-46°F use original vials only.
Number of determinations:	96 tests

14 PERFORMANCE DATA**14.1 Analytical Sensitivity**

Testing sample buffer 65 times with this ELISA gave a limit of blank of 0.53 U/ml and 10 low negative samples for 8 times gave a limit of detection of 1.69 U/ml.

14.2 Method Comparison

The microplates are coated with synthetic, deamidated gliadin-derived peptides. No cross reactivity with other autoantibodies have been found.

A total of 208 adult and pediatric samples (for composition see table) have been tested with this DGP-IgA and a predicate device reacting in the reportable range. Results are summarized in the following table (samples out of reportable range were excluded from the comparison but were included in the clinical validation below):

DGP-IgA	DGP-IgA	Predicate	
Diagnosis	POS (>18)	POS	Total
CD	45 (61.6%)	51 (69.9%)	73
CD IgA Def	0 (0%)	0 (0%)	2
CD suspect	23 (63.9%)	21 (58.3%)	36
CD suspect IgA Def	0 (0%)	0 (0%)	1
DH	35 (85.4%)	35 (85.4%)	41
Controls (non-DH/CD)	1 (1.8%)	1 (1.8%)	55
Total	104 (50%)	108 (51.9%)	208

		predicate		
		POS (>20)	NEG (≤20)	Total
DGP-IgA	Pos (>18)	88	16	104
	Neg (≤18)	20	84	104
	Total	108	100	208

Positive agreement	95% C.I.	
81.48% (88/108)	73.12%	87.68%
Negative agreement		
84% (84/100)	75.58%	89.91%
Overall Agreement		
82.69% ((88+84)/208)	76.97%	87.23%

(*) Agreements were calculated regarding equivocal results as negative and low positive results as positive.

Of the 36 samples with discrepant results this DGP-IgA outperformed the predicate device in 16 cases based on additional information such as EMA, biopsy and results from DGP assays of other immunoglobulin classes.

14.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly with a negative serum according to CLSI EP06-A. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

Composition		High			Medium			Low		
Pos. sample	Neg. sample	Mean [U/ml]	Expected [U/ml]	Recovery [%]	Mean [U/ml]	Expected [U/ml]	Recovery [%]	Mean [U/ml]	Expected [U/ml]	Recovery [%]
100.0%	0.0%	331.9	331.9	100.0%	89.6	89.6	100.0%	13.9	13.9	100.0%
87.5%	12.5%	297.8	290.4	102.5%	74.2	78.4	94.7%	10.9	12.2	89.5%
75.0%	25.0%	247.9	248.9	99.6%	68.0	67.2	101.2%	10.3	10.4	98.4%
67.5%	32.5%	203.5	224.0	90.8%	55.2	60.4	91.2%	9.2	9.4	98.0%
50.0%	50.0%	168.0	165.9	101.3%	39.4	44.8	88.0%	6.1	7.0	87.1%
37.5%	62.5%	141.5	124.4	113.7%	32.9	33.6	98.0%	4.8	5.2	92.1%
25.0%	75.0%	88.6	83.0	106.7%	18.7	22.4	83.5%	3.1	3.5	90.2%
12.5%	87.5%	35.6	41.5	85.7%	5.3	11.2	46.9%	1.3	1.7	73.4%

Taken this data, the linear range for this DGP-IgA ELISA ranges from 1.74 U/ml to 300 U/ml.

14.4 Precision

To determine the precision of the assay, the variability (intra, inter-assay and Lot-to-Lot) was assessed by examining its reproducibility on five serum samples selected to represent a range over the standard curve, in 8 repetitions in 5 runs. Lot-to-Lot variability was assessed measuring five serum samples in 8 repetitions on 3 different lots

Inter-assay variability			Intra-assay variability			Lot-to-Lot variability		
Sample No.	Mean (U/ml)	CV (%)	Sample No.	Mean (U/ml)	CV (%)	Sample No.	Mean (U/ml)	CV (%)
1	2.5	20.9	1	2.5	11.2	1	2.6	17.6
2	12.9	10.5	2	12.9	6.9	2	13.0	9.1
2b	16.7	10.7	2b	16.7	9.6	-	-	-
3	49.9	10.6	3	49.9	4.7	3	52.3	8.2
4	97.2	10.2	4	97.2	5.4	4	98.5	9.0
5	209.3	14.2	5	209.3	5.7	5	225.6	10.8

Acceptance criteria are $\leq 15\%$ for positive samples, $\leq 15\%$ for equivocal samples and $\leq 25\%$ for negative samples

14.5 Calibration

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

14.6 Normal Range

DGP-A antibodies are reported in up to 10% of the normal population.

133 random blood donors were tested for DGP-A antibodies. Of these; 116 ranged in the age of 16-45 and 17 were 46+; they included a similar number of males and females. One sample (0.8%) was equivocal, the rest were negative. The mean value of the samples was 3.1 units with a standard deviation of 1.8 units. The mean value is 8.5 standard deviations below the 18-unit limit of positivity.



15 LITERATURE

- Schwartz E, et al.:** Serologic assay based on gliadin-related nonapeptides as a highly sensitive and specific diagnostic aid in celiac disease. *Clinical Chemistry* 50: 2370-5, 2004.
- Osman AA, et al.:** B-Cell epitopes of gliadin. *Clin Exp Immunol* 121: 248-254, 2000.
- Mothes T.:** Deamidated gliadin peptides as targets for celiac disease-specific antibodies. *Adv Clin Chem.* 44: 35-63, 2007
- Aleanzi M, et al.:** Celiac disease: Antibody recognition against native and selectively deamidated gliadin peptides. *Clinical Chemistry* 47: 2023-8, 2001.
- Richter T, et al.;** Determination of IgG and IgA antibodies against native gliadin is not helpful for the diagnosis of coeliac disease in children up to 2 years old. *J Pediatr Gastroenterol Nutr.* 55: 21-5, 2012
- Leffler DA, Schuppan D.:** Update on serologic testing in celiac disease. *Am J Gastroenterol.* 105: 2520-4, 2010.
- Kurppa K, et al.:** Antibodies against deamidated gliadin peptides in early-stage celiac disease. *J Clin Gastroenterol.* 45: 673-8, 2011.
- Vermeersch P, et al.:** Diagnostic performance of IgG anti-deamidated gliadin peptide antibody assays is comparable to IgA anti-tTG in celiac disease. *Clin Chim Acta.* 411: 931-5, 2010
- Prause C, et al.:** Antibodies against deamidated gliadin as new and accurate biomarkers of childhood coeliac disease. *J Pediatr Gastroenterol Nutr.* 49: 52-8, 2009
- Naiyer AJ, et al.:** Comparison of commercially available serologic kits for the detection of celiac disease. *J Clin Gastroenterol.* 43: 225-32, 2009
- Fasano, A.** Surprises from Celiac Disease. *Scientific American.* 2009
- Ivarsson, A., et al.:** Breast-feeding protects against celiac disease. *Am.J.Clin.Nutr.* 75, 914–921, 2002
- Lagerqvist, C., et al.:** Antigliadin immunoglobulin A best in finding celiac disease in children younger than 18 months of age. *J.Pediatr.Gastroenterol.Nutr.* 47, 428–435. 2008
- Liu, E. et al.:** Natural history of antibodies to deamidated gliadin peptides and transglutaminase in early childhood celiac disease. *J.Pediatr.Gastroenterol.Nutr.* 45, 293–300. 2007
- Losowsky, M.S.** A history of coeliac disease. *Dig.Dis.* 26, 112–120. 2008
- Maglio, M. et al.:** . Serum and intestinal celiac disease-associated antibodies in children with celiac disease younger than 2 years of age. *J.Pediatr.Gastroenterol.Nutr.* 50, 43–48. 2010
- Not, T. et al.:** Celiac disease risk in the USA: high prevalence of antiendomysium antibodies in healthy blood donors. *ScandJ Gastroenterol* 33, 494–498. 1998
- Salmi, T.T., et al.:** Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. *Gut* 55, 1746–1753. 2006
- Schuppan, D., et al.:** Celiac disease: from pathogenesis to novel therapies. *Gastroenterology.* 137, 1912–1933. 2009
- Vermeersch, P. et al.** Diagnostic performance of IgG anti-deamidated gliadin peptide antibody assays is comparable to IgA anti-tTG in celiac disease. *Clin.Chim.Acta* 411, 931–935. 2010

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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konfirmationskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità