

IGF-II RIA

Radioimmunoassay for quantitative Determination of

Insulin-like Growth Factor-II (IGFBP blocked)

für Forschungsanwendungen / for research use only
Not for use in diagnostic procedures.
zum Gebrauch durch Fachpersonal! / for professional use!



2-8°C



100



REF **R30**

























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Symbols / Symbole

according to DIN EN 980 and EDMA recommendations Standard News 6 2001

	Expiry date / Verfallsdatum / Date de péremption / Data di scadenza / Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Wažności / Lejárati idő / Doba expirácie / Doba expirace
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	Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže
	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno
	Catalogue Number / Bestellnummer / Numéro de référence / Numero di riferimento / Número de referencia / Número de referência / Referentienummer / Referencenummer / Katalognummer / Numer katalogowy / Katalógusszám / Katalógové číslo / Katalogové číslo
	Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk ó ó között / Skladujte v rozsahu / Skladujte v rozmezí
	Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů
	Keep away from sunlight / Nicht dem Sonnenlicht aussetzen
	Radioactive / radioaktiv
	Incubation time / Inkubationszeit
	Incubate at / Inkubation bei
	Centrifuge/ Zentrifugieren
	Mix tubes with a Vortex mixer/ Mix Röhrchen mit Vortex Mixer
	Aspirate liquid completely / Flüssigkeit vollständig absaugen
	all / alle
	Not / Nicht
	maximal Binding / maximale Bindung
	Total Counts / Total Counts
	Reconstitute in / Rekonstituieren in
	Sample / Probe
	DB / A / AB / P Buffer / Puffer
	C Tracer / Tracer
	B 1. Antibody

2.Ab	O	2 .Antibody
DILU BUF X		Dilute in Buffer X / Verdünnen in Puffer X
STD X	E-L	Standard X / Standard X
Control	M / N	Control Serum / Kontrollserum
MEASURE		Count radioactivity of all Tubes / Radioaktivität aller Röhren messen

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INTENDED USE

A radioimmunoassay for the quantitative measurement of levels of IGF-II in serum or plasma. Human growth factor measurements are used in the research of growth disorders involving the anterior lobe of the pituitary gland.

SUMMARY AND EXPLANATION

Insulin-like growth factors (IGF) I and II play a pivotal role in regulating the proliferation, differentiation and specific functions of many cell types (1-3). IGF-II has a molecular weight of 7469 daltons (5). Its major regulators are growth hormone (GH) and nutrition (6), although its production in specific tissues is affected by a multitude of tropic hormones and other peptide growth factors. In contrast to many other peptide hormones, IGFs are avidly bound to specific binding proteins (IGFBP). The seven classes of IGFBPs which are known at present (7,8,22) either bind IGF-I and IGF-II with similar affinities or show a preference for IGF-II (9,10).

A major problem of IGF-II measurement results from the interference of IGFBPs in the assay. Direct determinations in untreated serum samples (11) give false values because of the extremely slow dissociation of the IGF/IGFBP complexes during the assay incubation. Depending on the ratio IGF to IGFBP the following errors may occur:

- in samples with low IGF concentration, IGFBP-complexation will take place predominantly with the IGF tracer, thus leading to false-high results in a competitive RIA. Effect: Overestimation of low IGF levels.
- in samples with high IGF concentration, unmarked IGF from the sample will be predominantly complexed by IGFBPs and therefore withdrawn from measurement. Effect: Underestimation of high IGF levels.

Therefore, various techniques were applied to physically separate IGF-II from its binding proteins before measurement, including (a) size exclusion chromatography under acidic conditions, (b) solid-phase extraction and (c) acid-ethanol extraction (2,12,13). These techniques, however, are either inconvenient or time-consuming or give incomplete

and not-reproducible recoveries. The most widely used method is the acid-ethanol extraction (13,14) with a recovery of only 70-80 % of IGFBP-bound IGF as a result of co-precipitation. The absolute results of such an extraction are therefore false low (15). The extraction removes the IGFBPs only insufficiently and leads to reduction in sensitivity of the assay due to pre-dilution of the samples by the extraction procedure. Furthermore, the remaining IGFBP may still interfere in the assay. In addition, the acid-ethanol extraction is ineffective in specimens other than serum or plasma (e.g. cell culture media), in which determination of IGF is already difficult enough due to the fact that IGFBPs are frequently present at large excess.

To avoid these difficulties, an uncomplicated assay was developed, in which special sample preparation is not required before measurement. (except dilution and/or acidification in a specially-composed buffer system).

PRINCIPLE

In order to dissociate IGF-II from the IGFBPs, the samples must be diluted in an acidic buffer. The diluted samples are then pipetted into the assay tubes. The IGF-II antiserum containing an excess of IGF-I is dissolved in a buffer, which is able to neutralize the acidic samples. After the IGF-II antibody solution has neutralized the samples, the excess IGF-I occupies the IGF-binding sites of the binding proteins, thus allowing the measurement of free IGF-II. With this method, the IGFBPs are not removed, but their function and therefore their interference in the assay is neutralized.

Due to the extremely low cross-reactivity of the IGF-II antibody with IGF-I, excess IGF-I does not disturb the interaction of the first antibody with IGF-II or IGF-II tracer. In order to separate bound and free tracer, the immuno-complex (antigen-spec. antibody) is pelleted by centrifugation.

WARNINGS AND PRECAUTIONS

- 1. For research and professional use only.**
2. For *in-vitro* use only.
3. The acquisition, possession and use of the kit is subject to the regulations of the national nuclear regulatory authorities.
4. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

5. Before use, all kit components should be brought to **room temperature at 20 - 25°C**, if not indicated differently. Precipitates in buffers should be dissolved before use by thorough mixing and warming. **Temperature WILL** affect the assay. However, values for the patient samples will not be affected.
6. Do not mix reagents of different lots. Do not use expired reagents.
7. Reagents contain Sodium-Azide (0.02%) as preservative. Sodium-Azide is very toxic, therefore, R-Phrases: 28, 32, 50/53 and S-Phrases 28, 45, 60, and 61 must be considered.
7. Caution: This kit contains material of human and/or animal origin. Source human serum for the Control Serum provided in this kit was tested by FDA recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibody. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.
8. **Radioactivity** - Before ordering or using radioactive materials, it is necessary to take the appropriate actions to ensure compliance with national regulations governing their use. Local rules in each establishment, which define actions and behavior in the radioactivity working areas, should also be adhered to. The advice given here does not replace any local rules, instructions or training in the establishment, or advice from the radiation protection advisers. It is important to follow the code of good laboratory practice in addition to the specific precautions relating to the radionuclide I-125 used.

Iodine-125 has a radioactive half-life $T_{1/2}$ of 60 days and emits 35.5 keV gamma radiation, 27 – 32 keV x-rays and no beta radiation. Shielding is effectively done by lead, first half value layer is 0.02 mm lead, reduction to 10 % is made by 0.2 mm.

To reduce the radiation dose time spent handling radioactivity should be minimized (plan ahead), and distance from source of radiation should be maximized (doubling the distance from the source quarters the radiation dose).

Formation of aerosols, e.g. by improper opening and mixing of vials or pipetting of solutions which may cause minute droplets of radioactivity become airborne, is a hazard and should be avoided.

Solutions containing iodine should not be made acidic, because this might lead to the formation of volatile elemental iodine.

As some iodo-compounds can penetrate rubber gloves, it is advisable to wear two pairs, or polyethylene gloves over rubber.

For cleaning of contaminated areas or equipment, the Iodine-125 should be rendered chemically stable by using alkaline sodium thiosulphate solution together with paper or cellulose tissue.

General First Aid Procedures:

Skin contact: Wash affected area thoroughly with water at least 15 minutes. Discard contaminated cloths and shoes. See a physician.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids. See a physician.

Ingestion: If swallowed, wash out mouth thoroughly with water, provided that the person is conscious. Immediately see a physician.

The handling of radioactive and potentially infectious material must comply with the following guidelines:

The material should be stored and used in a special designated area.

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Avoid direct contact with these materials by wearing laboratory coats and disposable gloves.

Spilled material must be wiped off immediately. Clean contaminated areas and equipment with a suitable detergent.

Unused radioactive material and radioactive waste should be disposed according to the recommendations of the national regulatory authorities.

REAGENTS PROVIDED

1)	BUF AB	Acidification Buffer AB , ready for use, 12.5 ml
2)	BUF DB	Dilution Buffer DB , ready for use, 125 ml , use for reconstitution of E-L , and for Dilution of M&N + Samples
3)	BUF A	Assay Buffer A , ready for use, 30 ml , use for reconstitution of B+C+D+O
4)	1.Ab	1. Antibody B , lyophilized, 11 ml , (anti-hIGF-II). Contains rabbit IgG and recomb. hIGF-I antibody. Reconstitute with 11 ml Assay Buffer A
5)	Tracer	Tracer C , lyophilized, 11 ml , (¹²⁵ I-IGF-II) (< 3 µCi or < 110 kBq), Reconstitute with 11 ml Assay Buffer A.
6)	NSB	NSB D , lyophilized, 500 µl , Rabbit immunoglobulin for non-specific binding (NSB), Reconstitute with 500 µl Assay Buffer A
7)	STD E-L	Standards E-L , lyophilized, 500 µl , Standard values are between 0 – 50 ng/ml (0; 0.4; 0.9; 2; 4.5; 10; 22.5, 50 ng/ml). Use 100 µl / tube. Reconstitute in 500 µl Dilution Buffer DB .
8)	Control	Control Serums M & N , lyophilized, 100 µl : Contain human serum. Concentration and the acceptable range are given on the certificate. Reconstitute with 100 µl Aqua dest. Further dilution according to sample dilution with Dilution Buffer DB (e.g. 1:101)
9)	2.Ab	2. Antibody O , lyophilized, 1 ml Reconstitute in 1 ml Assay Buffer A . Transfer dissolved material to reagent P immediately before use. The assay is unaffected by the possible occurrence of turbidity in the final reagent.
10)	PRE	Precipitation Reagent P , ready for use after adding O, 55 ml

Note: Ensure that lyophilized materials are completely dissolved on reconstitution. It is recommended to keep reconstituted reagents at **room temperature** for **half an hour** and then to mix them vigorously with a Vortex mixer. This is important in particular for the **controls M & N!**

MATERIALS REQUIRED BUT NOT PROVIDED

- 1) Pipettes: 10 ml, 500 µl, 250 µl, 100 µl, 10 µl, 25 µl, 100 µl, and 250 µl repeating pipettes are recommended.
- 2) Vortex mixer
- 3) Disposable polystyrene or polypropylene tubes. Conical tubes are highly recommended because of the small immunoprecipitates.

The use of round-bottom tubes may cause formation of insufficiently compact pellets.

Ice cold deionised water

- 4) Centrifuge appropriate for precipitation of immunocomplexes
- 5) Device for aspiration of liquids (e.g. connected to a water pump).
- 6) Gamma counter

STORAGE CONDITIONS

Store the kit at **2-8°C** after receipt until its expiry date. The lyophilized reagents should be stored at **-20 °C** after reconstitution. Avoid repeated thawing and freezing. The shelf-life of the reagents after opening is not affected, if used appropriately.

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

The stability of IGFBP-bound IGF-II makes sample preparation simple. Serum and EDTA plasma levels are comparable. Blood samples may be taken at any time of the day. Whole blood should be processed within a few hours and stored frozen at -20°C until measurement. IGF-II levels are usually not affected by improper handling or storage. They remain stable over several days in normal and in various clinical situations even under conditions of high temperature (37°C). Avoid repeated freezing and thawing cycles, although IGF-levels in normal sera remained unchanged after 10 cycles. Frozen samples are stable over many years. Samples may also be freeze-dried without suffering any loss of activity.

Sample requirements: 10 µL serum or plasma (minimum 5 µL).

Serum or plasma samples should be diluted 1 to 30-400 fold (or even more) depending on the expected values with the Dilution Buffer. Usually, a dilution of 1:100-150 is appropriate. For routine applications we recommend a dilution of 1:101.

Example: Add 10 µL serum to 1 ml **Dilution Buffer DB** (dilution 1:101).

If very low levels are expected (e.g. in extreme GH deficiency or in GH receptor deficiency), serum or plasma samples can be measured with 1:20 or lower dilution, they have to be acidified only. Sufficient acidification (pH≤3) can be achieved by adding 1/10 volume **Acidification Buffer AB** to the samples.

Example: Dilute 10 µL serum with 200 µL Dilution Buffer (1:21). Add 20 µL Acidification Buffer (total dilution 1:23).

The dilution of the controls **M & N** with **Dilution Buffer DB** should be according to the common dilution of serum or plasma samples, e.g. about 1:101

In body fluids other than serum or plasma (e.g. cerebrospinal fluid, ocular vitreous fluid, or urine) or in conditioned cell culture media IGF-II concentrations may be extremely low. These samples can be directly measured without dilution after adding 1/10th of their volume Acidification Buffer.

ASSAY PROCEDURE

Flow Chart of Assay Protocol:

#	Tube	DB	E-L M, N Patients	D	B	C	P
1,2	Total	–	–	–	–	100	–
3,4	NSB	100	–	100	–	100	500
5,6	B ₀	–	100 E	–	100	100	500
7-20	Standards	–	100 F-L	–	100	100	500
21,22	Control 1	–	100 M	–	100	100	500
23,24	Control 2	–	100 N	–	100	100	500
25,26	Sample 1	–	100	–	100	100	500
27,28	Sample 2	–	100	–	100	100	500
etc.							

Note: All volumes are given as µL.

Samples (standards and patient samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the test-protocol are recommended.

1) Labelling of the assay tubes should be done in the following order:

- 1, 2 total counts, **TC**
- 3, 4 non-specific binding, **NSB**
- 5, 6 zero standard **E (B₀)**
- 7, 8 standard **F**
- 9, 10 standard **G** etc.
- 21, 22 high control **M**
- 23, 24 low control **N**
- 25, 26 etc.duplicates of samples.

2) Add **100 µl** of Dilution Buffer **DB** to tubes 3 and 4.

- 3) Add **100 µl** of reagents **E - L** (standards) to tubes 5 to 20, (zero standard (E) to tubes 5 and 6, standard F (0.4 ng/ml) to tubes 7 and 8, etc).
- 4) Add **100 µl** of diluted reagent **M** (high control) to tubes 21 and 22 and diluted reagent **N** (low control) to tubes 23 and 24.
- 5) Add **100 µl** of diluted (or only acidified) **samples** to tubes 25 and 26, etc.
- 6) Add **100 µl** reagent **D (NSB)** to tubes 3 and 4.
- 7) Add **100 µl** reagent **B** (1st Antibody) beginning with tube 5.
- 8) Add **100 µl** reagent **C** (tracer) to all tubes.
- 9) Remove tubes 1 and 2 (total counts) or mark or seal with a stopper.
- 10) **Mix tubes** with a vortex mixer.
- 11) Incubate tubes at **2 - 8 °C** for **2 days**. Incubation of 1 day or more than 2 days will also be appropriate.
- 12) Add **500 µl** reagent **P** (after addition of reagent **O**!), beginning with tube 3. The reagent should be cold (2-8 °C).
- 13) **Mix tubes** with a vortex mixer.
- 14) Incubate tubes at **2 - 8 °C** for **1 hour**
- 15) Add **1 ml** ice-cold distilled water.
- 16) **Centrifuge** all tubes except tubes 1 and 2 at least at **3000 x g** for **30 min** at a temperature of **2 - 8 °C**.
- 17) **Aspirate** the supernatant (except tubes 1 and 2!). The remaining supernatant should not be higher than 2 mm above the precipitate. Take care that the precipitate remains intact.
Depending on local conditions and procedures, the supernatant may also be decanted instead of aspirated.
- 18) **Count the activity of all tubes (including tubes 1 and 2) for 1 to 3 min.**

Extended washing procedure for increased precision

The second incubation step (step 14) is directly followed by step 16 (centrifugation) and step 17 (aspiration). Proceed then with step 15 and add 1 ml of ice-cold water. This should not be done too vigorously in order to keep the precipitate intact. Do not mix again! Centrifuge the tubes at 2-8°C at 3000 x g for 5 min., aspirate the supernatant, and count the radioactivity of all tubes in the gamma-counter (step 18).

This extended procedure results in a somewhat higher precision and reduces the non-specific binding NSB. This is also bound up with a higher work expenditure. The higher precision may be irrelevant for most measurements and should therefore be used only in special cases.

QUALITY CONTROL

The handling of radioactive and potentially infectious material must comply with Good laboratory practice (GLP). GLP requires that controls be run with each standard curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state or local standards/laws. All standards and kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

Assay Characteristics and Validation

The tracer is prepared through radio iodination of recombinant hIGF-II. The standards are derived from recombinant hIGF-II.

CALCULATION OF RESULTS

Establishing of the Standard Curve

The standards provided contain the following concentrations of IGF-II :

Standard	E	F	G	H	I	J	K	L
ng/ml	0	0.4	0.9	2.0	4.5	10	22.5	50

1. Calculate the average counts (AC) of each pair of tubes. This gives the values for B
2. Subtract the average counts (AC) of tubes 3 and 4 (non-specific binding NSB) from the mean counts of the standards, controls and patient samples. This gives the corrected values for B.
3. The corrected value from the zero standard E (tubes 5 and 6) is B_0 .
4. Calculate the percent bound (% B/ B_0):

$$\% B/B_0 = B/B_0 \times 100\%$$

- Plot $\% B/B_0$ versus the standard concentrations on either semi-logarithmic or logit-log paper. For convenience, it is recommended to use computer assisted data reduction programs.
- Quality control 1, calculate the non-specific binding NSB in %:
NSB / Total Counts TC x 100%
= (AC tubes 3 + 4 / AC tubes 1 + 2) x 100%.
It should be < 5% (%NSB/TC < 5).

Quality control 2, calculate the percent bound of zero standard E:

$$B_0 / \text{Total Counts TC} \times 100\%$$
$$= ((\text{AC tubes 5 + 6} - \text{AC tubes 3 + 4}) / \text{AC tubes 1 + 2}) \times 100\%$$

It should be > 30% ($\%B_0/TC > 30$).

Evaluation of sample concentrations:

Read the concentration value (abscissa) corresponding to the $\% B/B_0$ of the sample as in the example given below:

average counts of NSB: 1353 cpm

average counts of zero standard (B_0): 18183 cpm

average counts of sample: 10036 cpm

$$\%B/B_0 = (\text{cpm sample} - \text{NSB}) / (\text{cpm } B_0 - \text{NSB}) \times 100\%$$

$$= (10036 - 1353) / (18183 - 1353) \times 100\%$$

$$= 0.516 \times 100\%$$

$$= 51.6 \%$$

For a 51.6 % value on the y-axis (ordinate) a value of 6.63 ng/ml on the x-axis (abscissa) was obtained. Multiply the concentration value determined graphically or by the aid of a computer programme with the dilution factor.

Example: $6.63 \times 101 = 670 \text{ ng/ml}$.

If it is preferred to express the results as nmol/l, the values given as ng/ml should be divided by 7.469 to obtain nmol/l.

Example: $670 \text{ ng/ml} / 7.469 = 89.7 \text{ nmol/l}$

Concentration of control samples

The IGF-II concentration of Control M & N should be within certified concentration range.

EXPECTED VALUES

Table 1: Serum levels of IGF-II in ng/ml in healthy subjects at various ages*

Percentile			
Age group	5th	50th	95th
Newborns	158	284	516
1-4 weeks	350	486	673
1-6 months	348	551	871
6-12 months	388	582	876
1-3 years	384	596	926
3-5 years	397	617	920
5-7 years	419	638	973
7-9 years	433	656	997
9-11 years	442	662	994
11-13 years	448	671	1006
13-15 years	455	679	1014
15-17 years	452	686	1042
20-30 years	436	679	1058
30-40 years	442	680	1049
40-50 years	407	650	1039
50-60 years	396	644	1049
60-70 years	373	611	1000

* Measurement was performed after acid-ethanol extraction, and values were corrected for recovery (correction factor 1.2). Blum W., Schweizer R.,: Insulin-like growth factors and their binding proteins, in Ranke MB (ed): Diagnostics of endocrine function in children and adolescents. Basel, Karger, 2003, pp 166-199 (22).

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity of the radioimmunoassay for IGF-II R30 yields 0.1 ng/ml 2x SD of zero standards.

Specificity

	IGF-I
Reactivity [%]	0.05

Reproducibility

Intra-Assay-Variation

	Number of determinations	Mean value (ng/ml)	Standard deviation (ng/ml)	CV (%)
Sample 1	8	630	5.9	0.9
Sample 2	8	226	5.2	2.3
Sample 3	8	613	9.1	1.5

Inter-Assay-Variation

The inter-assay variation coefficient at 50 % B/B₀ is 4.0%

	Number of determinations	Mean value (ng/ml)	Standard deviation (ng/ml)	CV (%)
Sample 1	13	706	41.8	5.9
Sample 2	13	248	20.2	8.1
Sample 3	11	501	25.1	5.0

Linearity

Dilution:	Sample 1 (calculated, ng/ml)
1:80	781
1:125	801
1:200	892
1:320	865
1:500	787
AV / 1SD / VK	825 / 49.8 / 6.0

AV = Average Value , SD = Standard Deviation

VK= Coefficient of Variation

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Summary of the assay

Reagent preparation:	Reconstitution	Dilution
1. Antibody (B)	in 11 ml Assay Buffer (A)	
Tracer (C)	in 11 ml Assay Buffer (A)	
NSB (D)	in 500 µl Assay Buffer (A)	
Standards (E-L)	in 500 µl Dilution Buffer (DB)	
Controls (M+N)	in 100 µl distilled water	1:101 with DB
2. Antibody (O)	in 1 ml Assay Buffer (A)	
Mix immediately before use with 55 ml Reagent (P)		
Dilute Sample with Dilution Buffer (DB) e.g. 1:101		

Assay procedure in double determination

Addition of Reagent [µl]						
Nr. of Tubes	Contents of Tubes	DB (Dilution Buffer)	E-L (Standards) M,N (Controls) SAMPLES	D (NSB)	B (1.Antibody)	C (Tracer)
1,2	Total	–	–	–	–	100
3,4	NSB	100	–	100	–	100
5,6	B ₀	–	100 E	–	100	100
7-20	Standards	–	100 F- L	–	100	100
21,22	High Control	–	100 M	–	100	100
23,24	Low Control	–	100 N	–	100	100
25,26	Sample 1	–	100	–	100	100
27,28	Sample 2	–	100	–	100	100
etc.						
Nr.:1,2 remove until counting the activity.						
Mix other tubes with a Vortex-Mixer.						
Incubation at 2-8°C, 2 days						
Add 500 µl P (after addition of reagent O) in all Tubes (except 1,2). The reagent-mix should be cold (2-8°C).						
Mix with Vortex-Mixer.						
Incubation at 2-8°C, 1 h						
Add 1 ml cold A.dest. carefully in all tubes (except 1,2)						
Centrifugation at ≥ 3000 x g, 30 min at 2-8°C						
Aspirate the supernatant (as a precaution e.g. ca. leave 2 mm as a remaining supernatant above the precipitate).						
Count the activity of all tubes with a Gamma counter.						

Exemplary version - do not use for assay accomplishment!
Exemplarische Version - nicht zur Assaydurchführung benutzen!


REF R30 

International Test description

Control	M & N	Rec in 100 µl A.dest	1:101 DILU BUF DB
1.Ab	B	Rec in 11 ml BUF A	
2.Ab	O	Rec in 1 ml BUF A	1:56 DILU BUF P
STD	E-L	Rec in 500 µl BUF DB	
NSB	D	Rec in 500 µl BUF A	
Tracer	C	Rec in 11 ml BUF A	
SPE + Control 1:101	DILU BUF DB		100 µl
°C 20-25 °C			

Tube		BUF DB	STD E-L	NSB D	1.Ab B	Tracer C
1/2	Tracer C = TC	-	-	-	-	100 µl
3/4	NSB	100 µl	-	100µl	-	100µl
5/6	B₀ STD E (0 ng/ml)	-	100 µl	-	100 µl	100 µl
7-20	STD F-L	-	100 µl	-	100 µl	100 µl
21/22	CONTROL N 1:101 DILU BUF DB	100 µl	-	-	100 µl	100 µl
23/24	CONTROL M 1:101 DILU BUF DB	100 µl	-	-	100 µl	100 µl
25...	SPE 1:101 DILU BUF DB	100 µl	-	-	100 µl	100 µl


↔ **ALL** **NOT** **TC**

 >48 h **°C** 2-8

500µl **2.Ab** **O** **DILU** **BUF** **P** **°C** 2-8 ↔ **ALL** **NOT** **TC**

 1 h **°C** 2-8

1 ml **A. dest** **°C** 2-8 **ALL** **NOT** **TC**

 ≥ 3000× g 30 min **°C** 2-8

ASP **ALL** **NOT** **TC**

MEASURE