

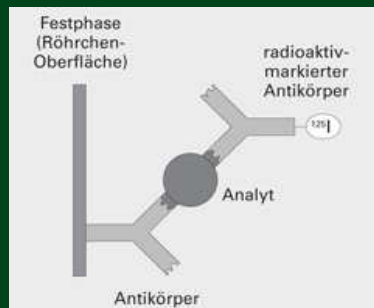
DYNE



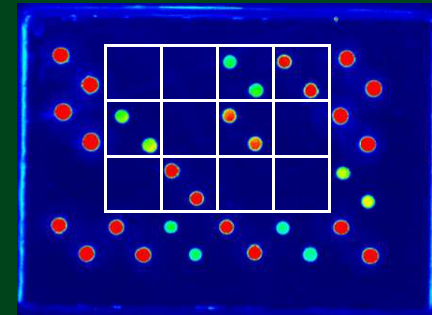
EUROIMMUN Product Range

- **Designer Antigens (e.g. rec. PR3, GAF-3X)**
- **Recombinant Cell-based IFT (e.g. PLA2-R, NMDA-R)**
- **Other applications than antibody detection (e.g. Vitamin D)**

RIA



DNA Microarrays

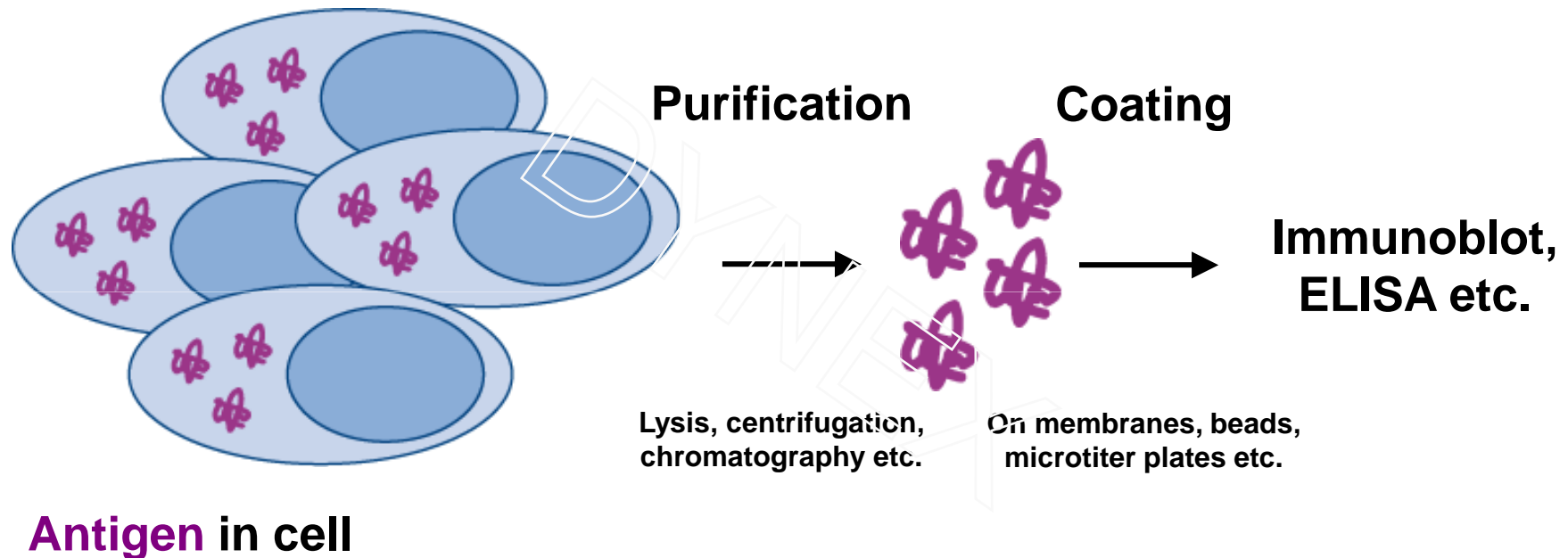


Recombinant Cell-based IFT:

**Phospholipase A2 Receptor Autoantibodies in
Idiopathic Membranous Nephropathy**



Use of recombinant antigens in monospecific assays



**If purification of antigens is difficult
cell-based assays are a fast alternative !**



Why can purification be a problem ?

1) Antigen contains fragile conformational epitopes:



3D
structure:
Ab binds



Linear structure:
Ab does not bind

Antigen must be purified with
intact 3D-structure
→ complex and time-consuming

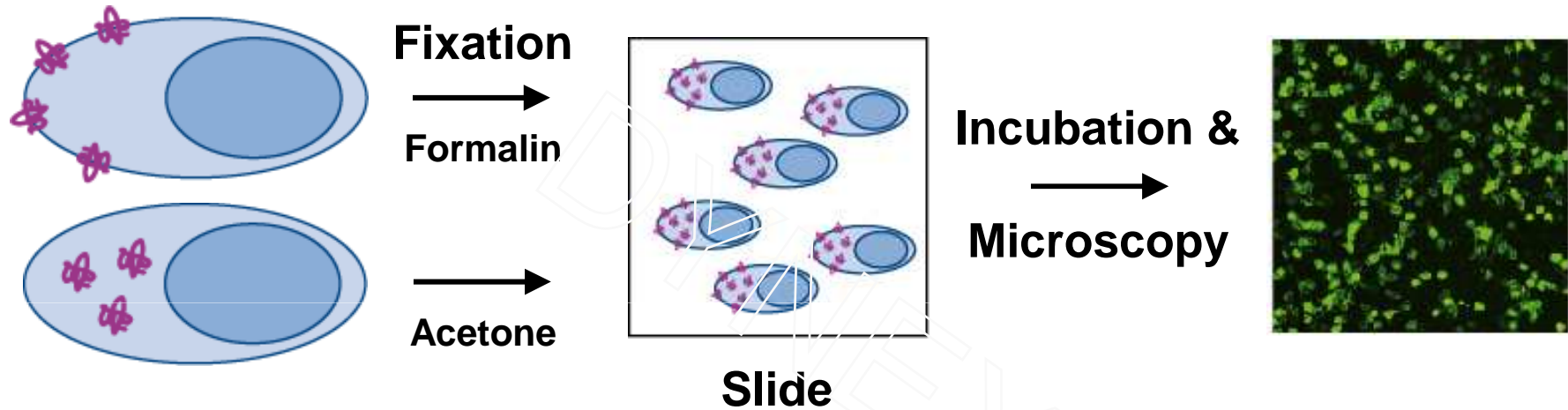
2) Antigen is membrane-bound:



Connection with the membrane is very stable
→ low yield, problematic purity
→ detergents are necessary for isolation
→ may result in the destruction of the epitopes



Recombinant Cell-based IFT

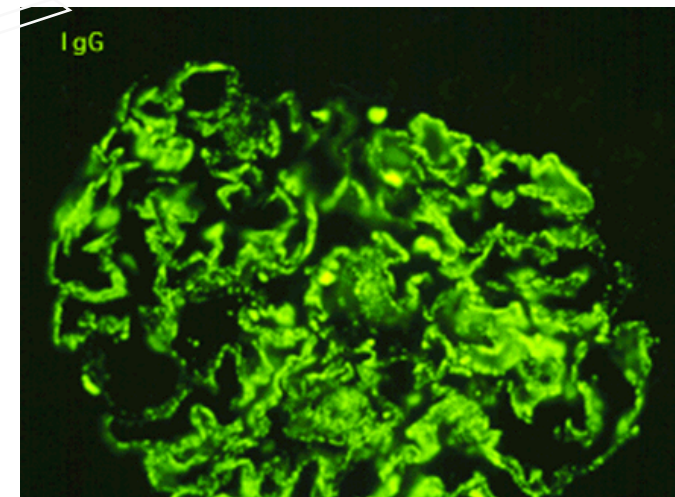


- Large-scale generation of recombinant cell substrates
- Antigen-dependent fixation of transfected cells
- Untransfected cells serve as a negative control



Membranous Nephropathy

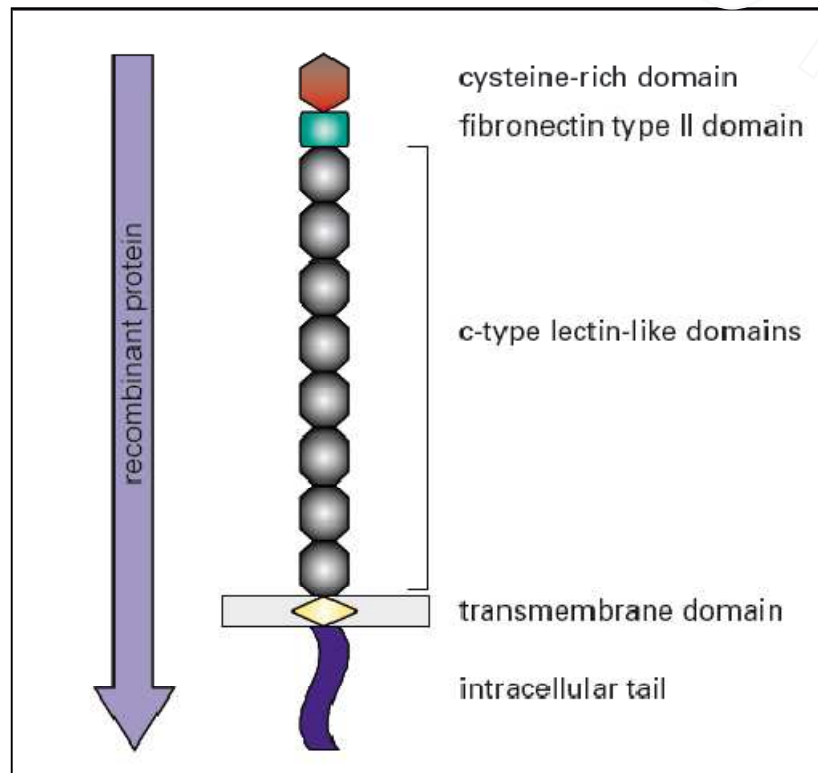
- Chronic glomerulonephritis by subepithelial immune complex formation resulting in a progressive impairment of kidney functions (⇒ nephrotic syndrome)
- Alters permeability of the capillary loops leading to proteinuria
- Most frequent cause of a nephrotic syndrome in adults, with a peak between the age of 30 - 40 and 50 – 60
- Male to female ratio is 3 : 1
- Idiopathic or primary form (70 – 80%)
- Secondary form in other diseases
 - Carcinoma
 - Hepatitis B
 - SLE
 - Diabetes mellitus
 - Medication-induced



Idiopathic Membranous Nephropathy: - Anti-PLA2 Receptor -

Beck et al.: M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy.

N Engl J Med. 2009 Jul 2;361(1):11-21



“Up to 70% of patients with idiopathic membranous nephropathy (IMN) exhibit autoantibodies against conformational epitopes on the phospholipase A2 receptor type M (PLA2R).“

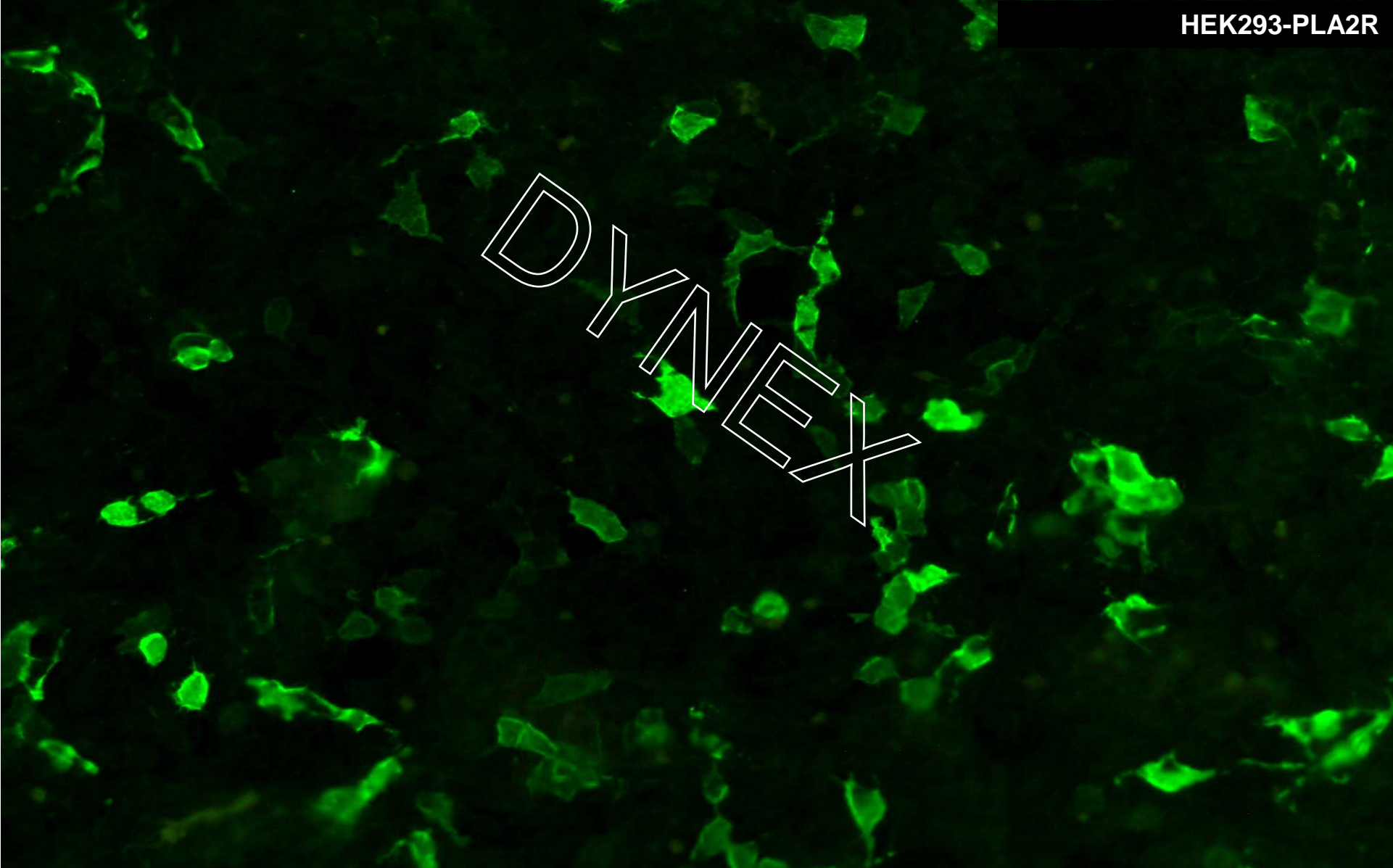


Anti-PLA2-R recombinant cell-based IFT

Cooperation: Prof. Stahl, University Hospital Eppendorf, Hamburg

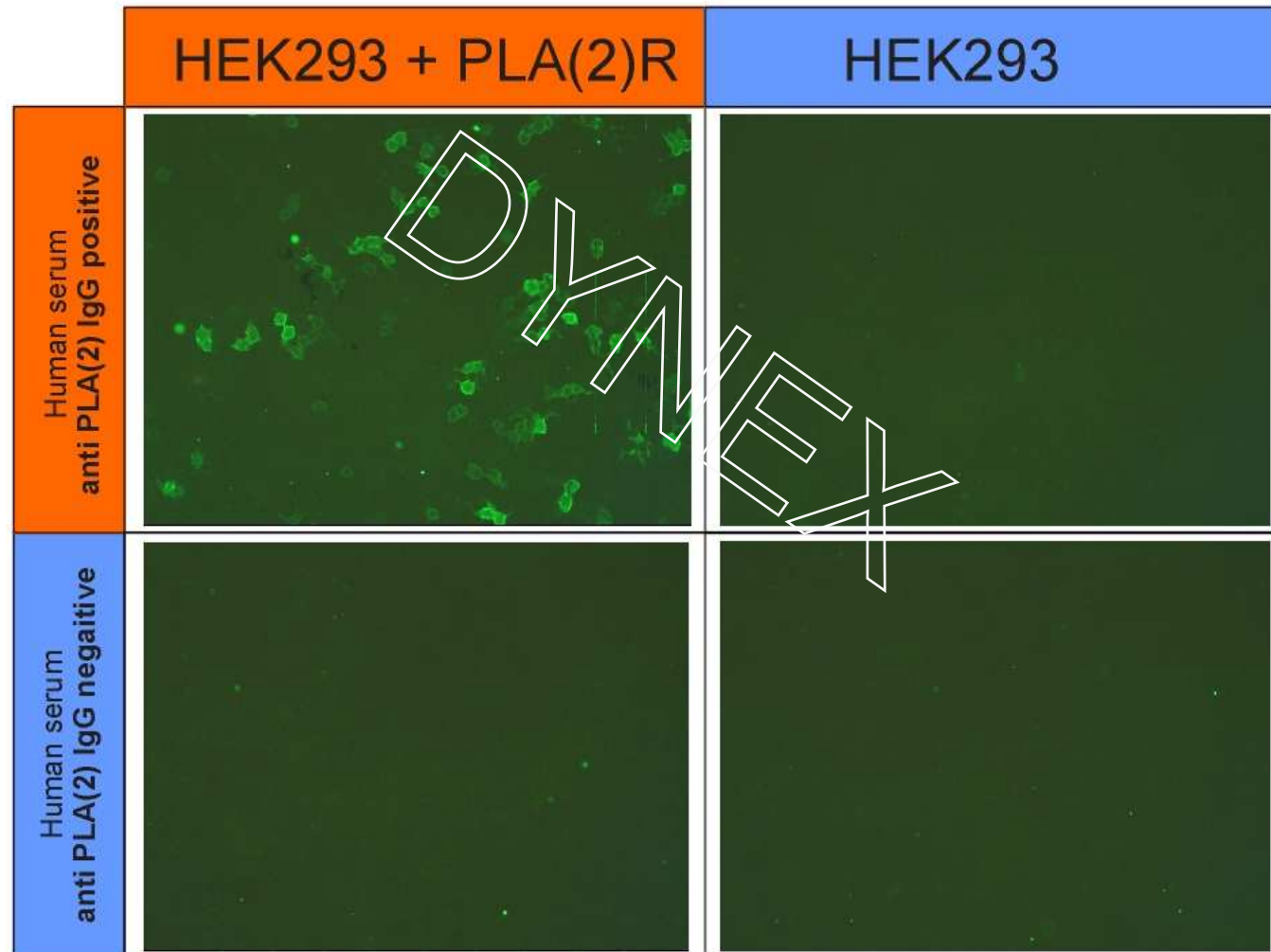
HEK293-PLA2R

DYNEEX

A fluorescence microscopy image showing a dense population of HEK293-PLA2R cells. The cells exhibit bright green fluorescence, primarily concentrated in the cytoplasm and nucleus. The background is dark, highlighting the individual cells. The word 'DYNEEX' is overlaid in the center of the image in a white, outlined, sans-serif font, rotated diagonally.

Anti-PLA2-R recombinant cell-based IFT

Cooperation: Prof. Stahl, University Hospital Eppendorf, Hamburg



Anti-PLA2-R IFT

- Sensitivity and Specificity -

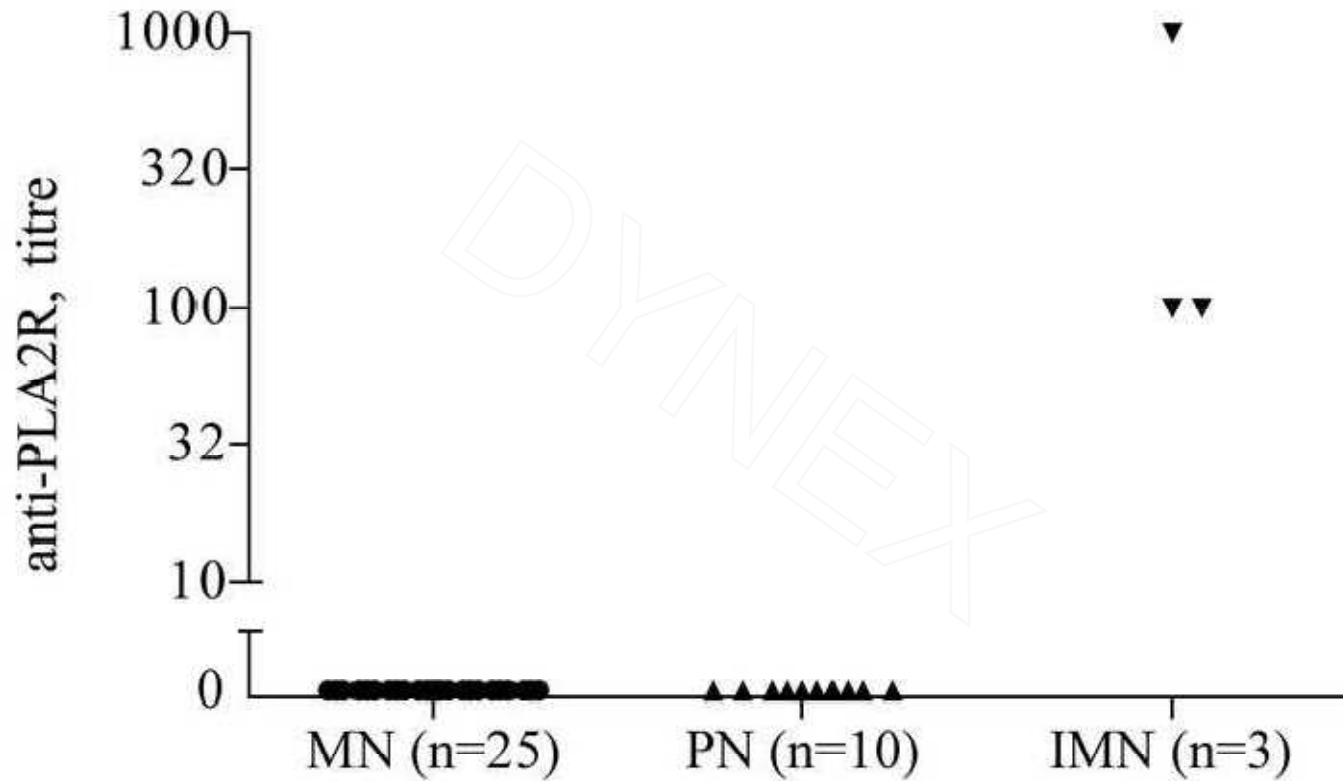
Cohorts (n = 360)	n	Anti-PLA2R positive
Biopsy-proven MGN	100	52
Secondary form of MGN	17	0
Non-membranous glomerulonephritis	90	0
Healthy blood donors	153	0

Sensitivity: 52%

Specificity: 100%



Anti-PLA2-R IFT - Specificity -



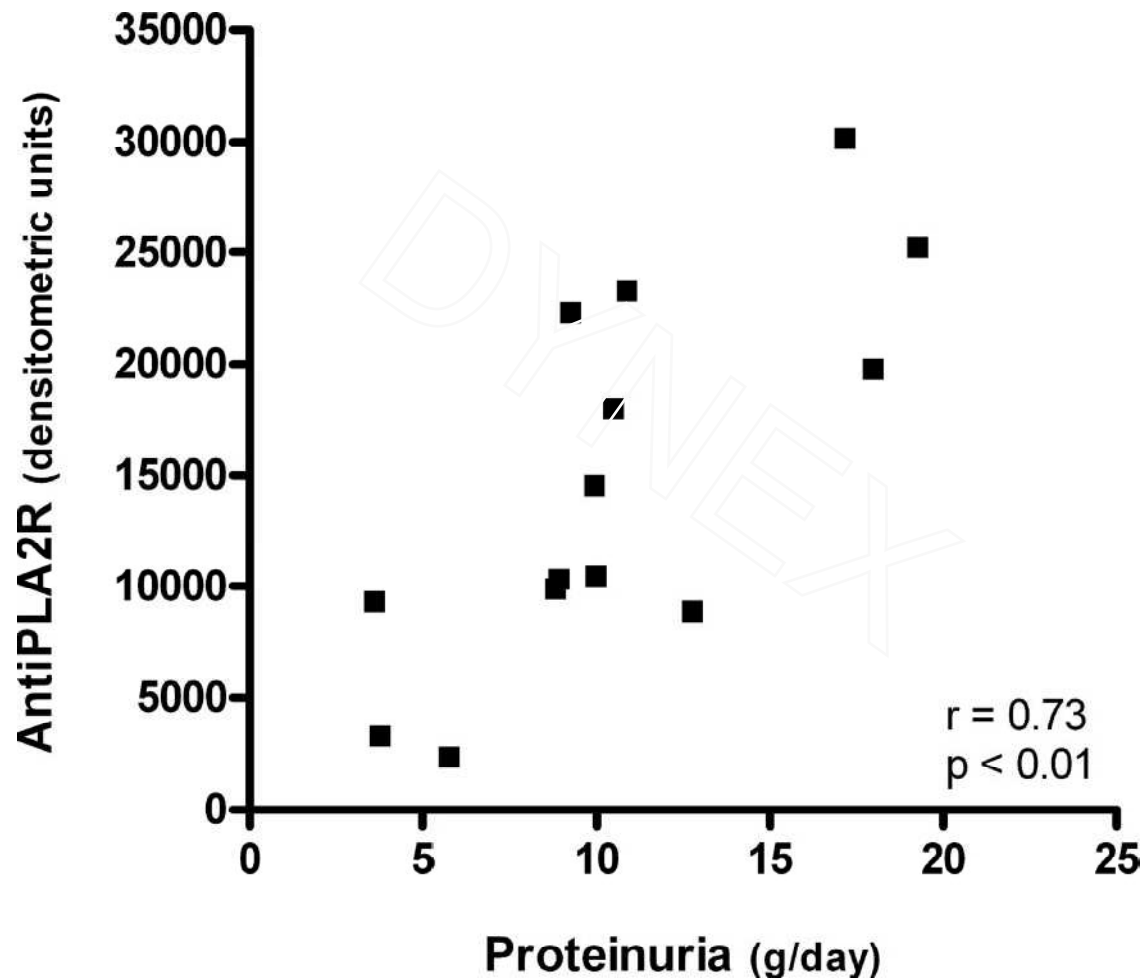
MN = Membranous Lupus Nephritis

PN = Proliferative Lupus Nephritis

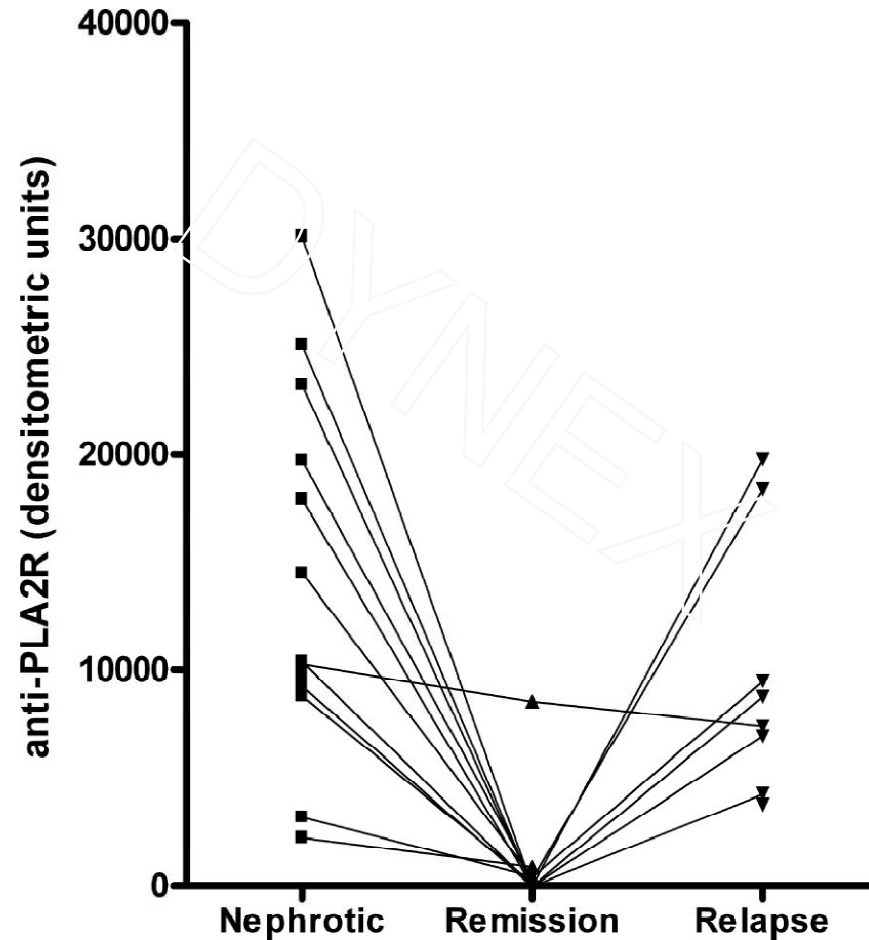
IMN = Idiopathic Membranous Nephritis



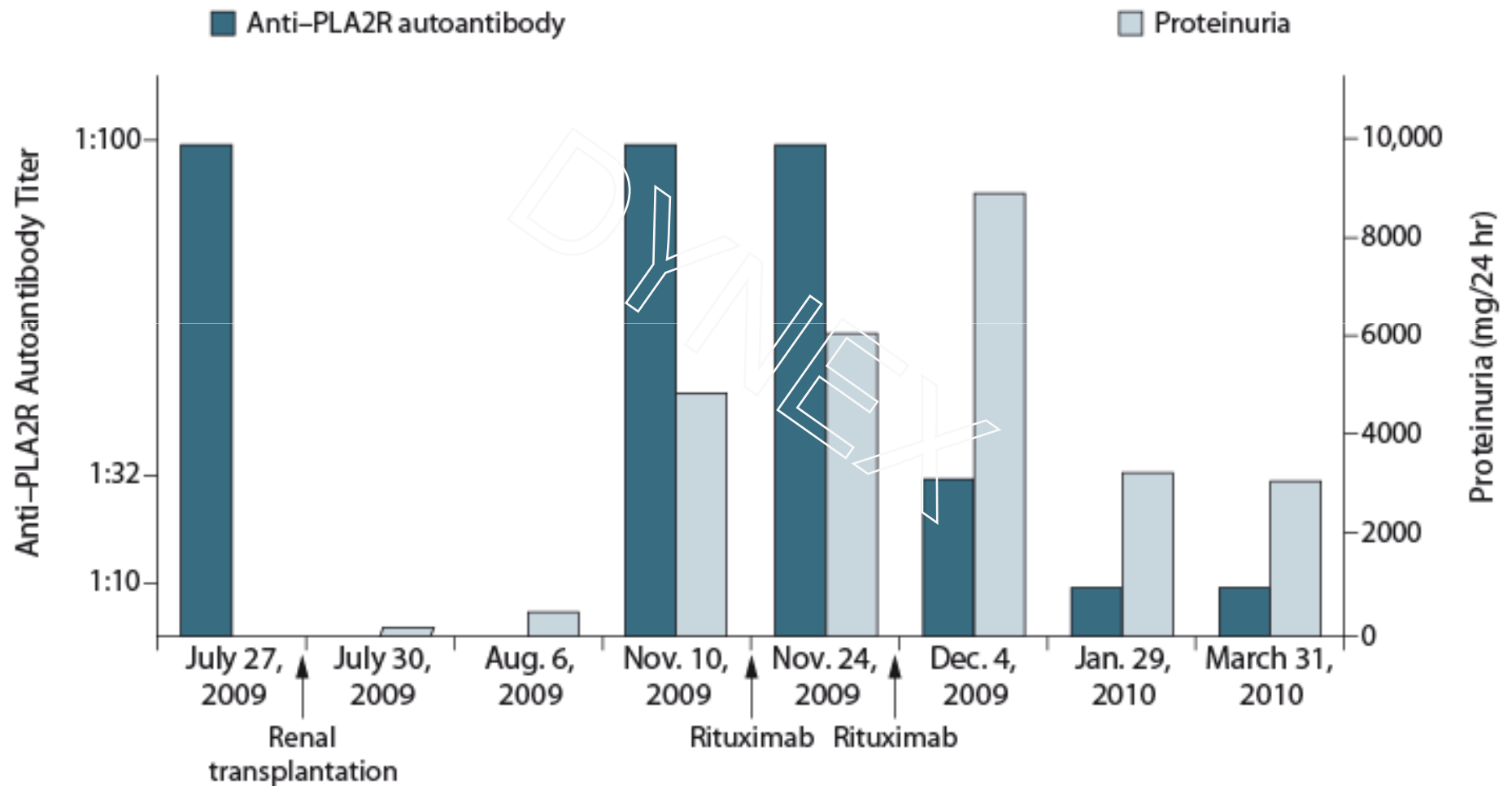
Anti-PLA2-R Titer and Clinical Presentation - Correlation to Severity of Disease -



Anti-PLA2-R Titer and Clinical Presentation - Monitoring the Clinical Status -



Anti-PLA2-R Titer and Clinical Presentation - Monitoring Response to Treatment -



Indication of anti-PLA2R testing

- **Differential diagnosis of nephrotic syndrome**
→ **primary idiopathic membranous nephropathy**
- **Assessment of disease activity and severity**
- **Monitoring response to treatment**



Vitamin D

The sunshine vitamin (pro-hormone)

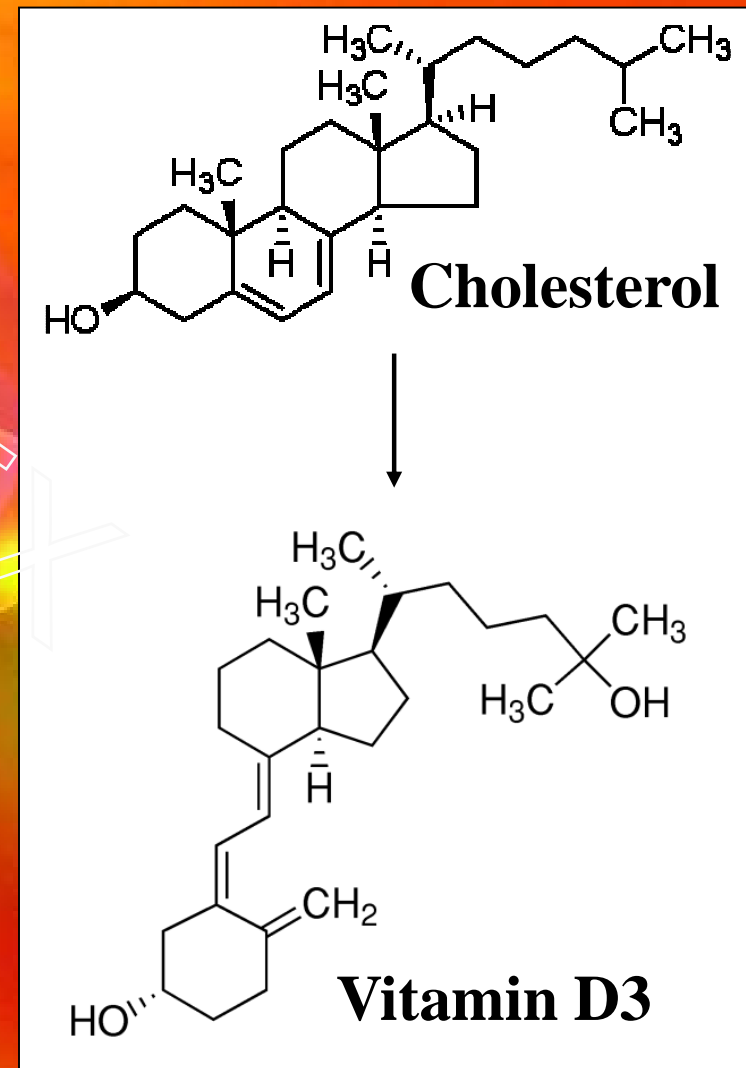
Cholesterol: precursor of Vitamin D synthesis

Vitamin D3: produced within the skin (under sunlight, UV-B), in food from animal origin

Vitamin D2: in some food from plant origin

25-OH Vitamin D3/D2: storage form of Vitamin D within the body (represents the long term vitamin D status)

1,25(OH)₂ Vitamin D3/2: active hormone (= Calcitriol)



Vitamin D: Functions (1)

Important role in calcium metabolism

- Enhances calcium and phosphate absorption in the gut and renal tubules
- Regulates the calcium concentration in the blood and bone growth

Bone development

- Calcium absorption (small intestine)
- Calcium resorption (bone and kidney)
- Maintain blood calcium levels
- Phosphor absorption

Cell differentiation

- Collagen and skin epithelium



Vitamin D: Functions (2)

Immunity (key hormone for the immune system)

- Cell mediated immunity and coordination of the immune response (T-cell stimulator)
- Avoids overreaction of the immune system and therefore the development of autoimmune diseases like Diabetes or Multiple Sclerosis

Hormone

- Regulation of gene expression
- Regulation of cell growth
(nearly all cell types in the body express a vitamin D receptor)

...



Vitamin D Deficiency Symptoms

Deficiency of vitamin D leads to :

- Rickets (in children)
- Osteomalacia (in adults)
- Lower bone density (higher risk for fractures)
- Osteoporosis



Vitamin D Deficiency Risks

Vitamin D Deficiency is also a risk factor for:

- Autoimmune diseases (Multiple sclerosis, Crohn's disease, diabetes mellitus, SLE, RA)
- Osteoporosis and Osteopenia
- Amyosthenia and Fibromyalgia
- Dementia and Parkinson
- Allergies
- Cancer
- Cardiovascular diseases, high blood pressure
- Higher susceptibility to infections
- Increased mortality
- ...

Optimal Vitamin D levels are essential for optimal health!



Vitamin D

Interpretation of serum concentrations

Serum concentration ng/mL	Interpretation
<10	Serious deficiency (rickets, osteomalacia !)
10-20	Deficiency (long term risk)
20-30	Suboptimal level
30-50	Optimal level
50-70	Higher normal level
70-150	Level too high (overdose), but not toxic
>150	Intoxication



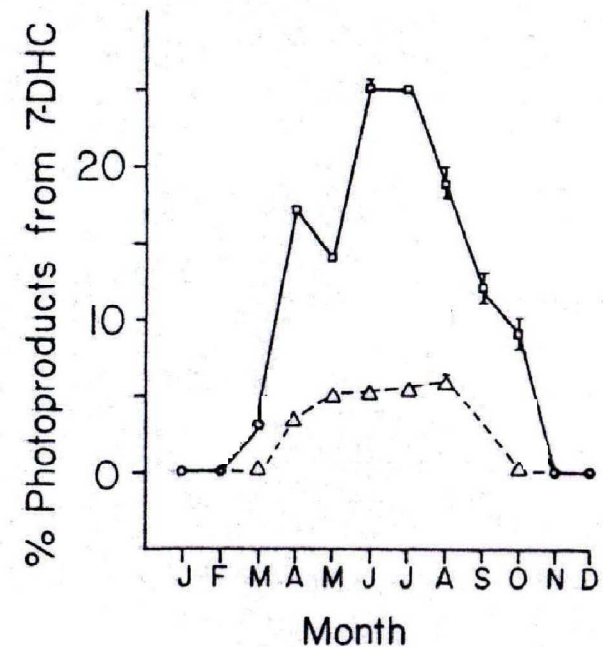
Vitamin D ELISA

Reasons for Deficiency

- Lack of sunshine exposure in modern society, lack of outdoor activities
- Lack of UV light in winter times
- Malnutrition or inadequate intake of Vitamin D
- Too much sunblockers
- Skin is completely coated with clothes

Risk groups:

- Kidney failure or chronic liver failure
- Infants
- Elderly
- Dark skinned
- Covered women





Vitamin D

Prevention and Deficiency Treatment

- Normal food does not contain adequate amounts of Vitamin D
- Not enough sun light (UV-B) in our latitude region
- More than one billion people worldwide have a Vitamin D deficiency, in some regions more than 50% of the population

Prevention (1 μg = 40 IU)

- Adding Vitamin D to foods (recommended intake of 5-10 μg per day for young children and adolescents; 10-20 μg for elderly; higher doses needed during pregnancy)
- Daily exposure to sunshine

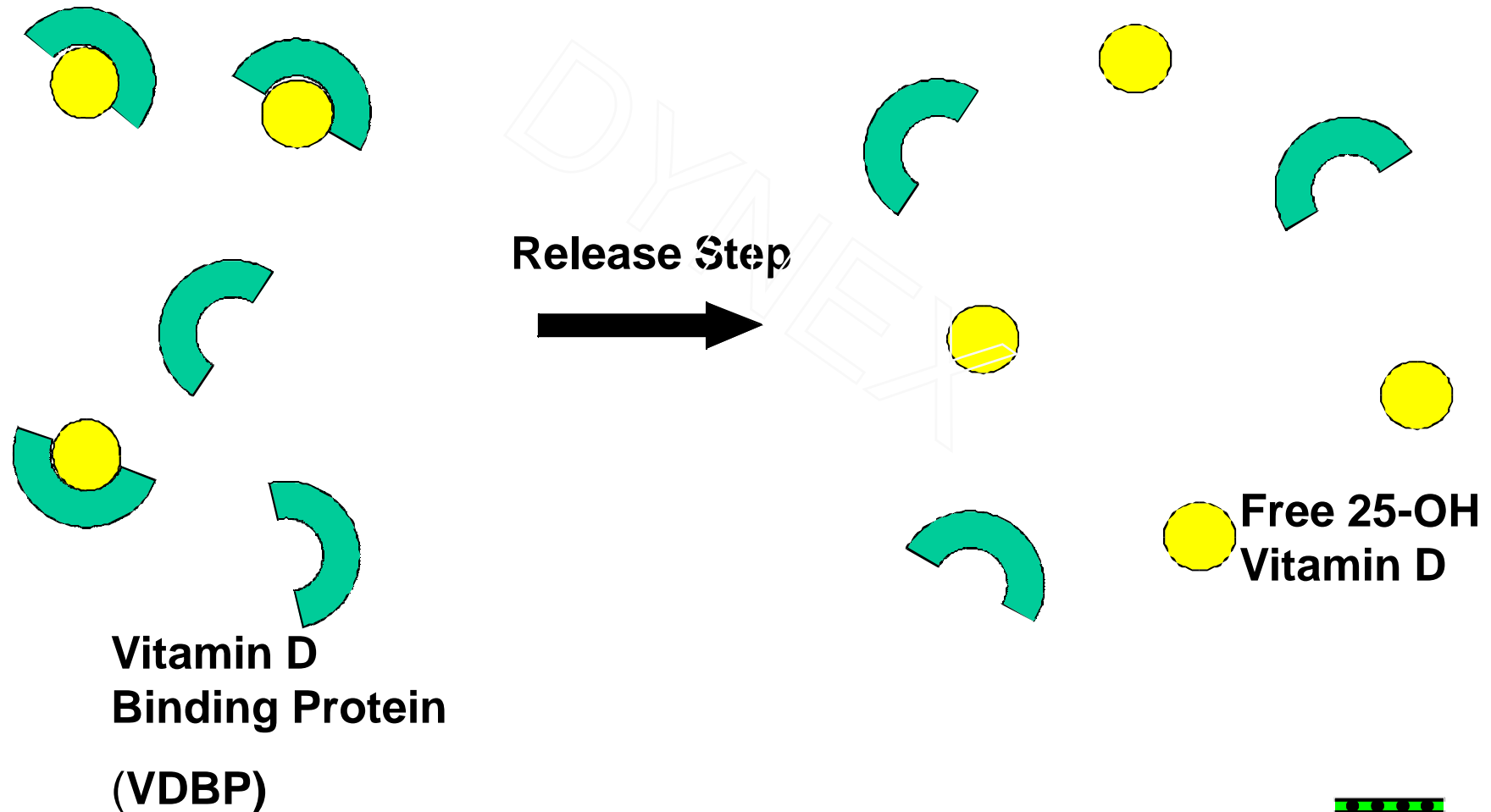
Deficiency treatment

- Vitamin D doses of 2.000-4.000 IU/day for several weeks
- Single large dose of 200.000-300.000 IU may be used monthly

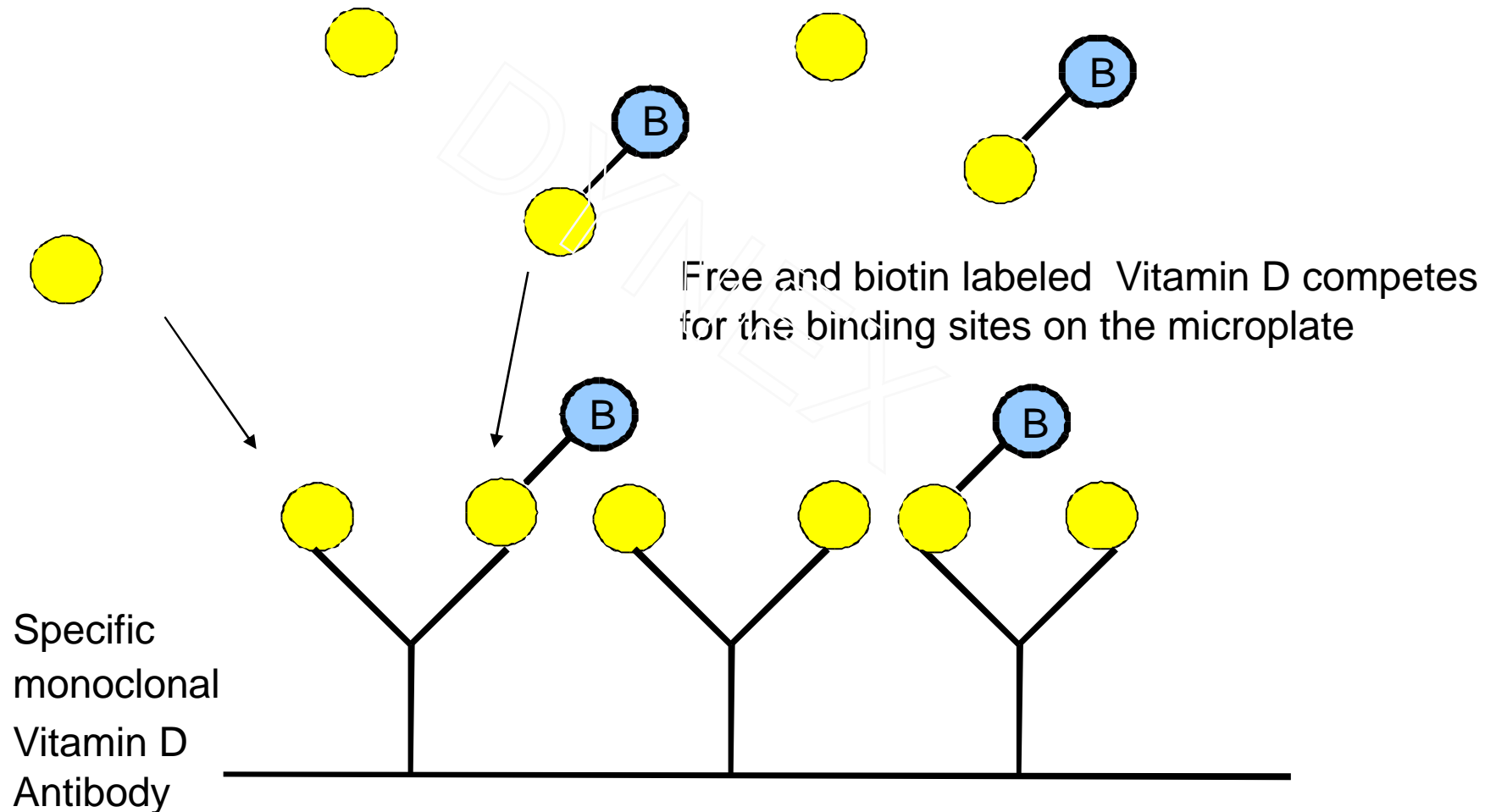


25-OH Vitamin D ELISA Principle (1)

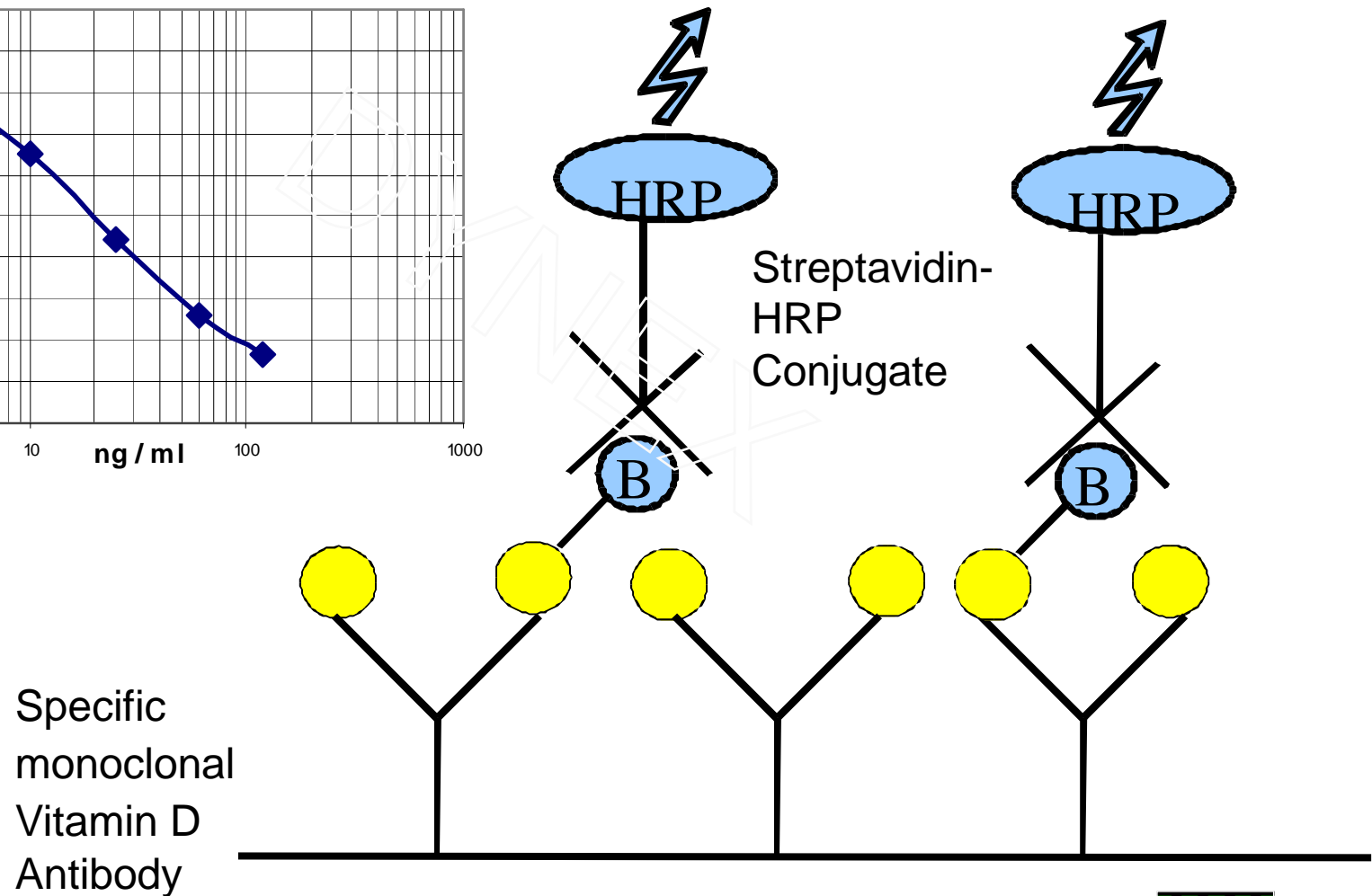
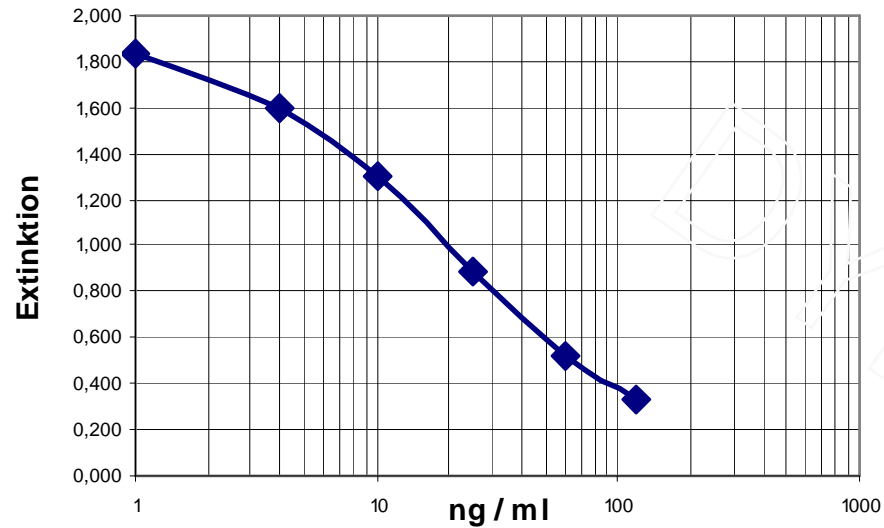
Vitamin D in the blood is bound to Vitamin D Binding Protein



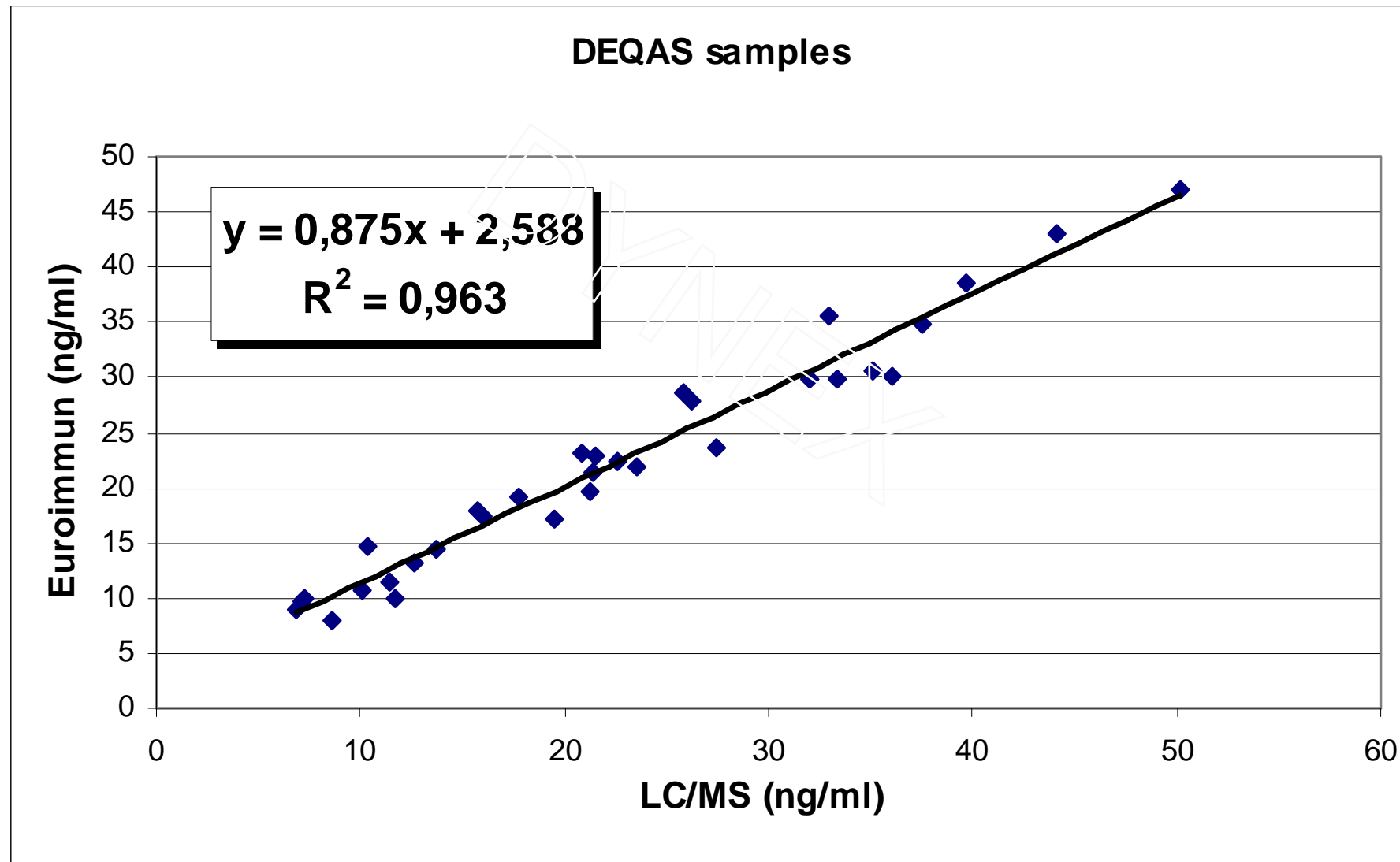
25-OH Vitamin D ELISA Principle (2)



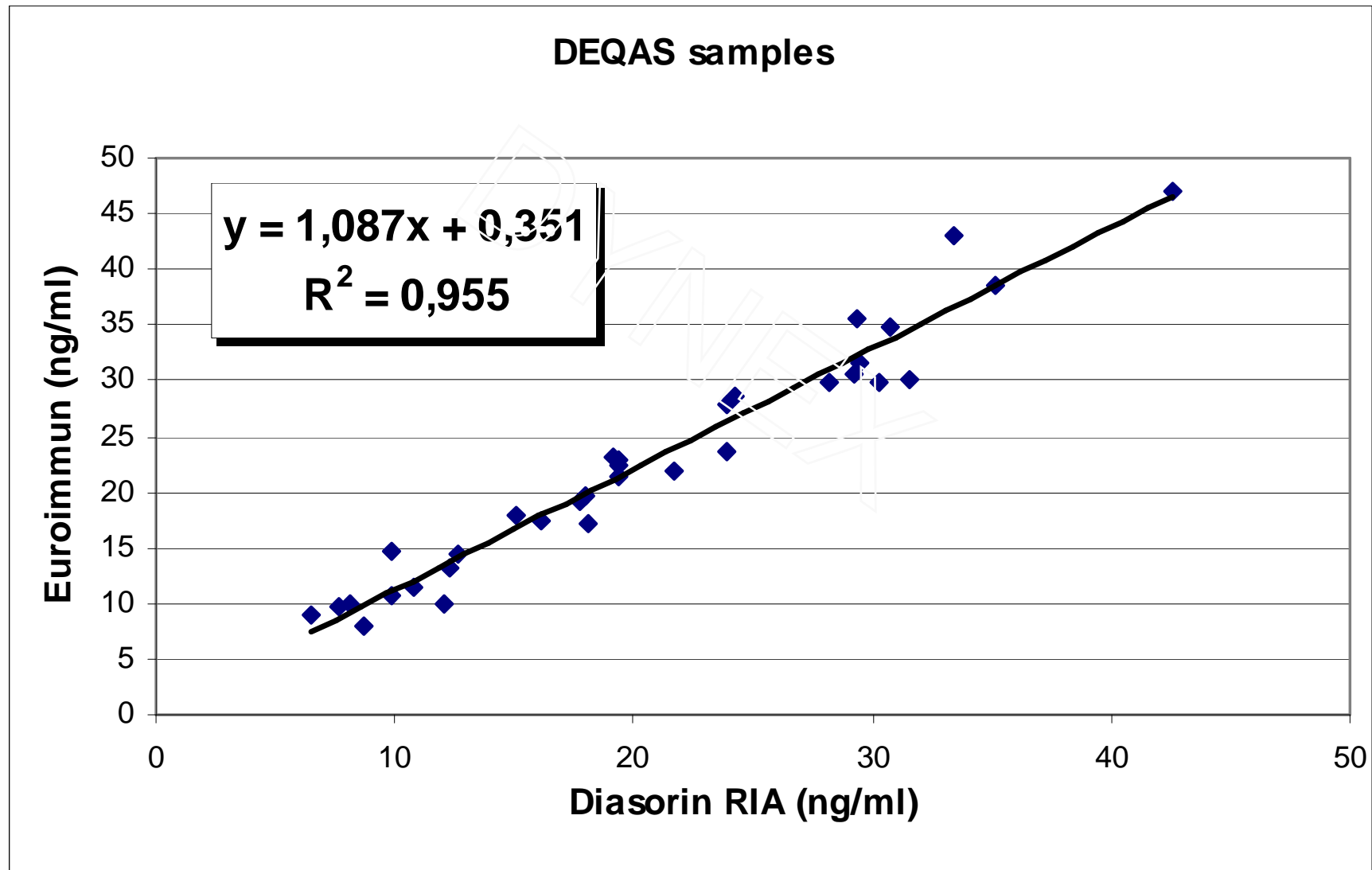
25-OH Vitamin D ELISA Principle (3)



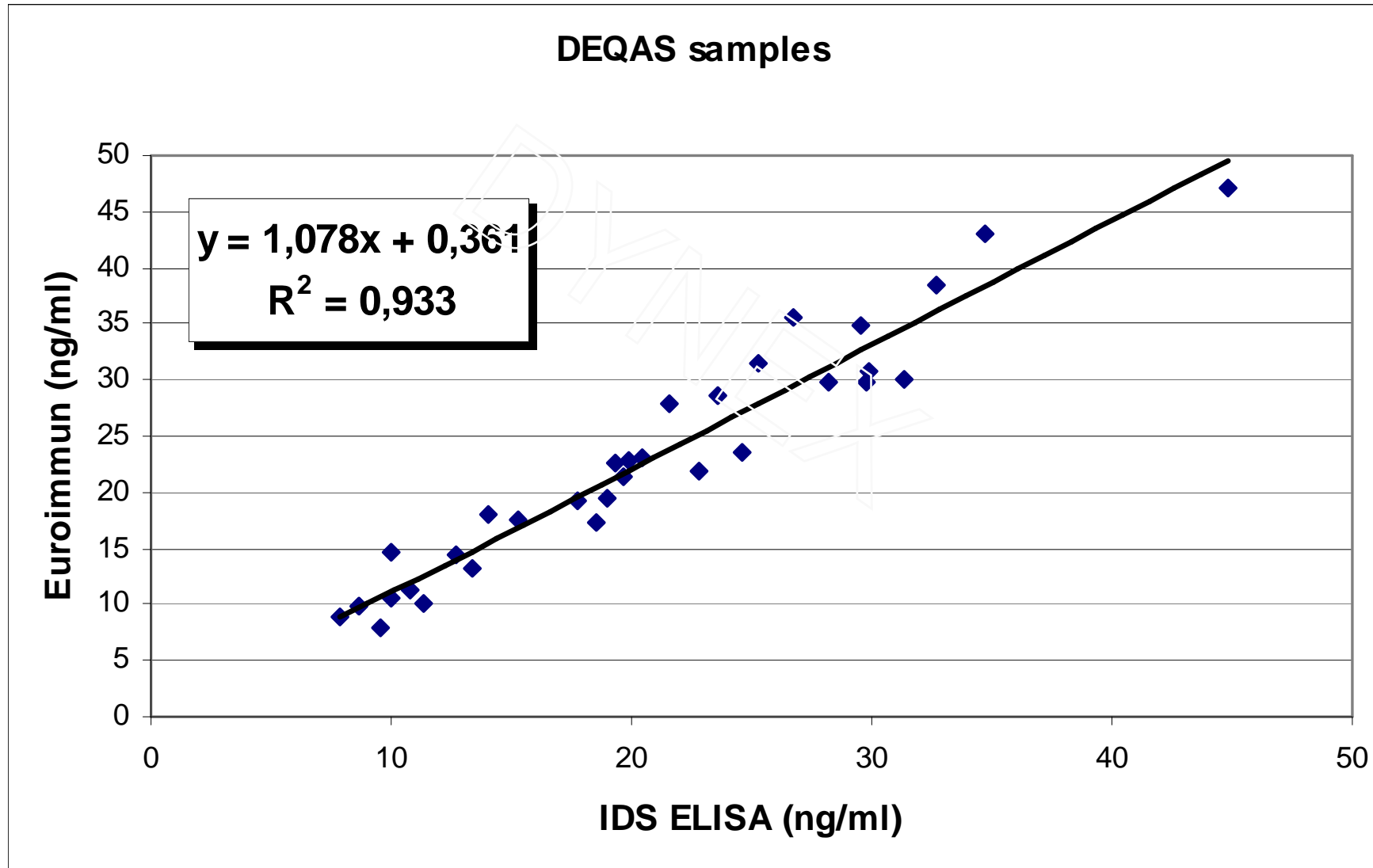
25-OH Vitamin D ELISA Correlation to HPLC



25-OH Vitamin D ELISA Correlation to RIA



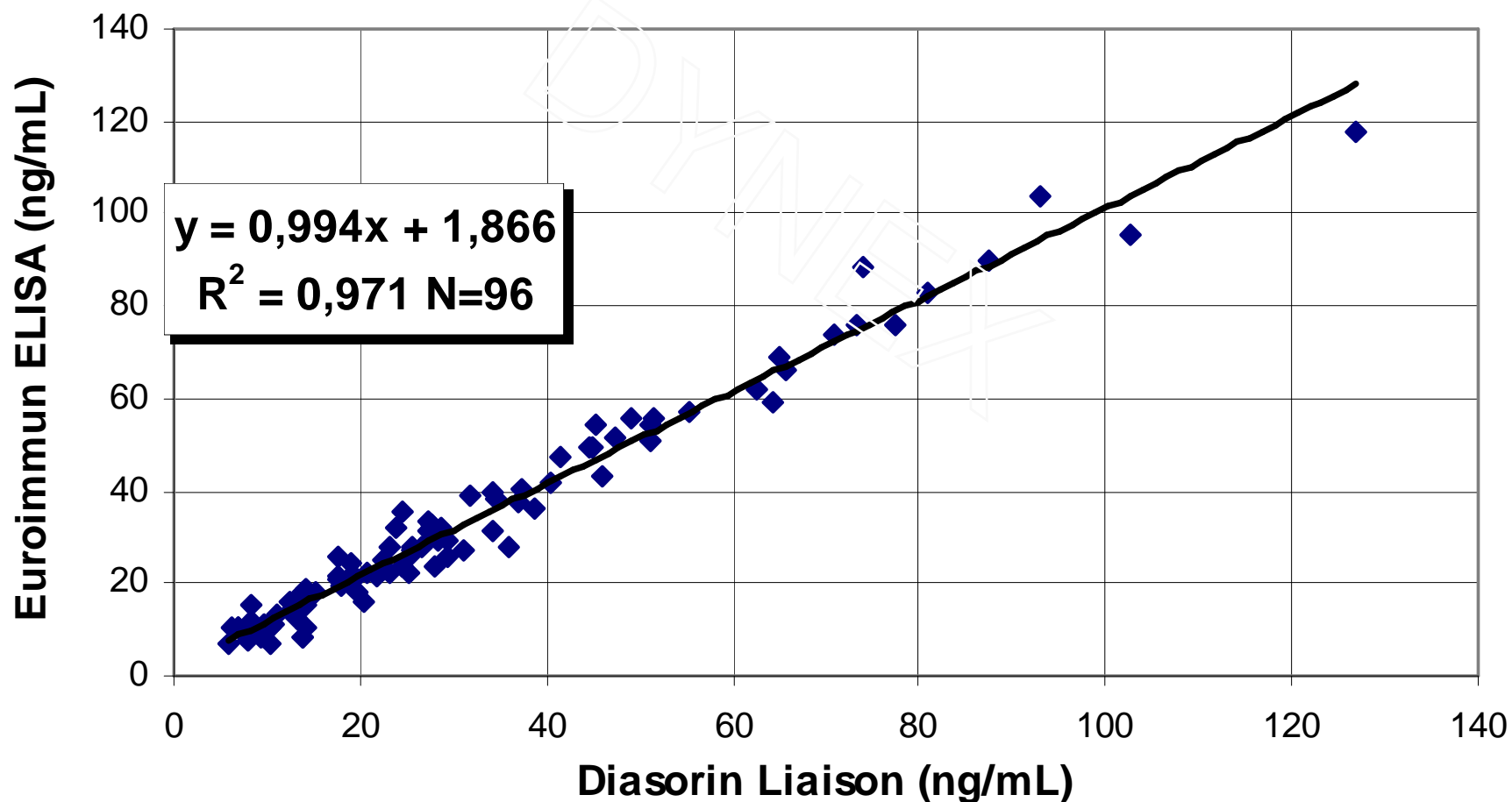
25-OH Vitamin D ELISA Correlation to ELISA



25-OH Vitamin D ELISA

Correlation to chemiluminescence assay

Method comparison 25-OH-Vitamin-D
Euroimmun vs. Diasorin Liaison



25-OH-Vitamin-D-ELISA

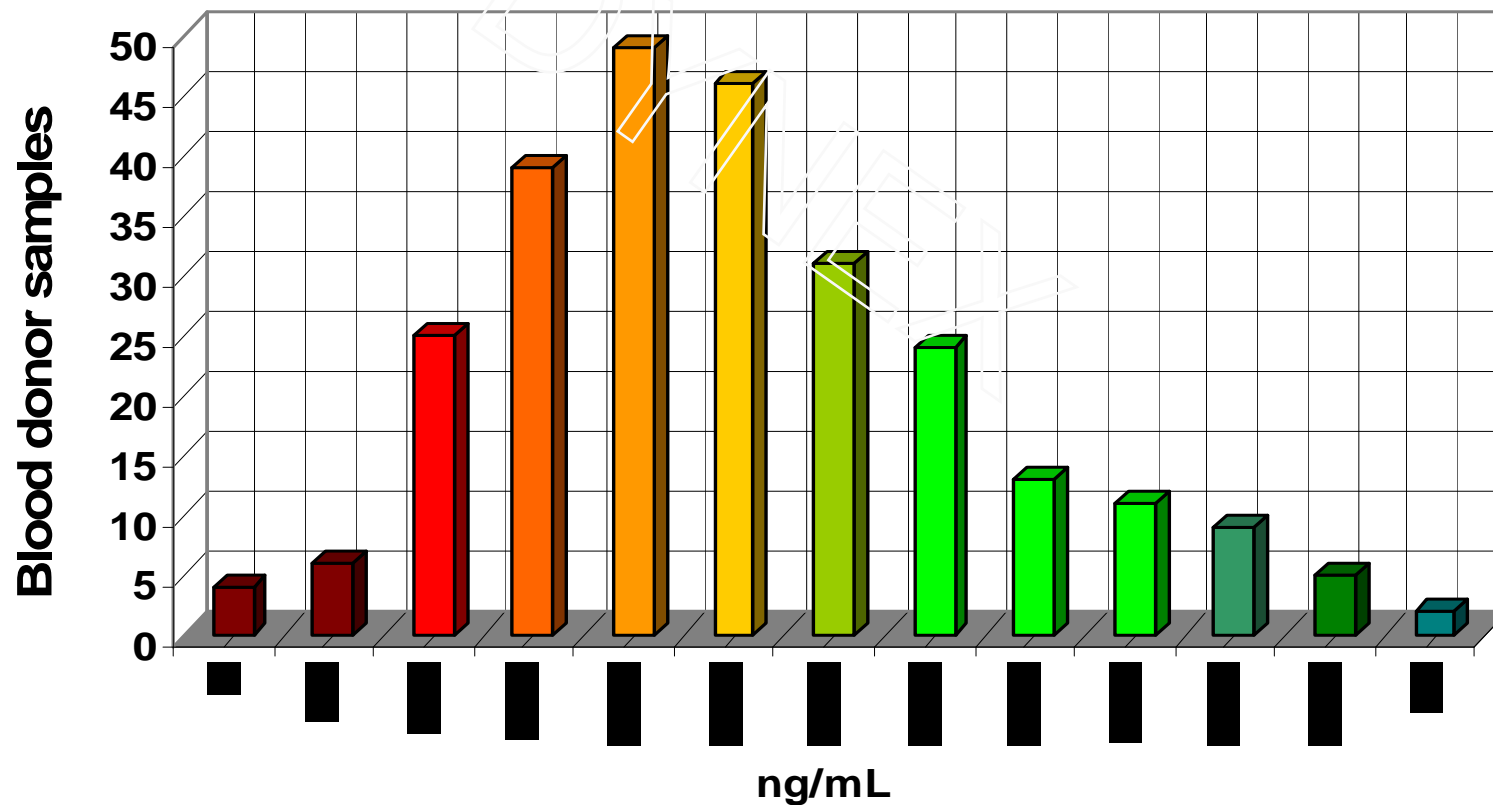
Measured values

Serum concentration ng/mL	Interpretation	354 blood donors (19 to 68 years)
<10	Serious deficiency	10
10-20	Deficiency (long term risk)	64
20-30	Suboptimal level	95
30-50	Optimal level	79
50-70	Higher normal level	14
70-150	Overdose, but not toxic	2
>150	Intoxication	0
Mean 28 ng/mL		

25-OH-Vitamin-D-ELISA

Measured values

25-OH-Vitamin-D
reference values, n=264



25-OH Vitamin D ELISA Advantages

- **New monoclonal antibody specific for 25-OH Vitamin D2 as well as D3**
- **Sample release in one step without toxic reagents**
- **6 standards and 2 controls included**
- **No lyophilized components, all reagents except wash buffer and biotin ready to use**
- **High correlations to established methods (LC/MS, IDS, Diasorin)**
- **Automated on analyzer with barcodes on each single reagent**



Do not disturb!

**Vitamin D
production
in progress!**



Do not carry a sunbath to excess! Or...



Horror dermatologicus

