Diagnostic Reagents Limited

UK CA

Immuno Depleted Human Plasma

Catalogue Numbers: IDHP180, 190, 191, 200, 210, 220, 230, 240, 250, 260

For *in vitro* diagnostic use only.

Intended Use

Diagen Immuno-Depleted Human Plasmas are designed for use as a substrate plasma in the quantitative determination of specific clotting factor concentration in plasma by clotting assay.

Diagen Factor V Depleted Plasma may also be used as substrate plasma for determination of Activated Protein C resistance. Please refer to the instructions for use regarding Diagen PCA ratio with factor V depleted plasma (APCV – IFU) for further information.

Summary and Principle

Diagen have developed a range of immuno-depleted human plasmas prepared by passing normal plasma over immobilised monoclonal antibodies The resulting plasma is depleted to less than 0.5% of the appropriate clotting factor whilst leaving other necessary clotting factors in adequate excess. When a patient plasma sample shows a prolonged Prothrombin Time (PT) or Activated Partial Prothrombin Time (APTT) it suggests that there may be a factor deficiency. If a patient's plasma is then mixed with a plasma known to be deficient in one specific factor (congenital or immunodepleted), the degree of correction of the clotting time is proportional to the level of this factor in the patient's plasma.

Reconstitution

For reconstitution, remove the cap and rubber stopper, add the appropriate volume of distilled water, as stated on the vial label and mix gently. Allow 5 to 10 minutes for complete solution.

Collection of Blood Samples

Blood (9 parts) is collected into 1 part of 3.2% trisodium citrate and the plasma obtained by centrifugation at 2500 g for 15 minutes. The plasma should be stored in stoppered tubes. The use of 3.2% citrate containing 5% HEPES buffer improves the stability of both fresh and deep-frozen plasma.

Warnings and precautions

POTENTIAL BIOHAZARD MATERIAL.

Diagen Immuno-Depleted Human Plasma is of human origin. All donor units used in production of this product have been found negative for anti-HIV, anti HCV, HBsAg and Syphilis by approved methods. However, all plasma of human origin should be considered as potentially infectious and handled appropriately. Please refer to the relevant SDS Sheet (provided on request) for handling and safety procedures. Dispose of all waste materials according to the stated international, national and local regulations.

Procedure

Materials Provided

This pack insert is applicable to the following immuno-depleted plasmas:

Cat. No.

IDHP180 - Fibrinogen depleted human plasma (10 x 1 mL vials).
IDHP190 - Factor V depleted human plasma (10 x 1 mL vials).
IDHP191 - Factor V depleted human plasma (10 x 2 mL vials).
IDHP200 - Factor VII depleted human plasma (10 x 1 mL vials).
IDHP210 - Factor VIII depleted human plasma (10 x 1 mL vials).
IDHP220 - Factor IX depleted human plasma (10 x 1 mL vials).
IDHP230 - Factor X depleted human plasma (10 x 1 mL vials).
IDHP240 - Factor XI depleted human plasma (10 x 1 mL vials).
IDHP250 - Factor XII depleted human plasma (10 x 1 mL vials).
IDHP260 - Protein C depleted human plasma (10 x 1 mL vials).

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Materials and equipment required, but not provided:

1. General routine laboratory coagulation equipment.
2. Diagen PT or APTT reagent and 25mM Calcium chloride.
3. Reaction cups or test tubes (12 x 75 mm).
4. Pipettes delivering between 50 µL and 1 mL.
5. Distilled water.
6. Imidazole Buffer (IMBX600) for making plasma dilutions.
7. Diagen Reference Control Plasmas: RCPN070 (Normal). RCPA080 (Abnormal).

Procedure

For PT based assays:

1. To a clotting tube, add 100 µL of factor deficient plasma to 100µL of standard (or test plasma) dilution.
2. Incubate at 37°C for 2 minutes.
3. Add 200 µL of Calcium Rabbit Brain Thromboplastin (prewarmed to 37°C) and start the clot timer.
4. Record the clotting time. Duplicates should be performed for each dilution.

For APTT based assays:

1. To a clotting tube, add 100 µL of factor deficient plasma to 100µL of standard (or test plasma) dilution.
2. Incubate at 37°C for 2 minutes.
3. Add 200 µL of Kaolin APTT reagent or 100 µL of Micronised silica APTT reagent.
4. Incubate at 37°C for exact time give in the reagent instructions for use.
5. Add 100 µL of 25mM Calcium Chloride and start the clot timer.
6. Record the clotting time. Duplicates should be performed for each dilution.

Notes:

1. Prepare a minimum of three dilutions of standard and test plasma in Imidazole buffer (1:10, 1:30 and 1:100). The results obtained with these dilutions must fall within the sensitive (linear) part of the curve. If they do not, prepare appropriate dilutions. For example, samples measured ≤10% can be tested at the 1:5 dilution, and obtained results divided by 2; for samples measured >100%, the 1:20 dilution can be used and obtained results multiplied by 2.

2. For photo-optical and mechanical instruments, follow the manufacturer's instructions.

Expression of results

Plot the log clotting time against the log concentration of the relevant clotting factor. The test results are applied in the same manner and the results obtained by interpolation from the 100% line (see figure 1). In this example the test has a value of antilog 1.38 or 24 % **of the standard value.**

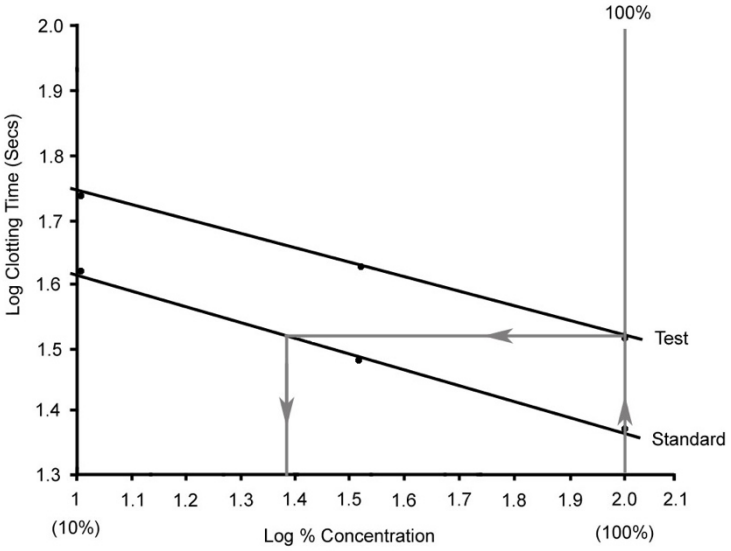

Figure 1. A graph illustrating the interpretation of results (example only).

Quality Control

All laboratories should have in place a quality control system that uses control plasmas titrated for factor activity to validate the calibration curve, as well as monitor both reagent and user performance. We recommend using Diagen Reference Control

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Plasma – Normal (RCPN070) as a standard, alongside an Abnormal (RCPA080) as a consistent control on the test system. If the controls do not perform within their reference ranges, further investigation into the assay system may be required.

Limitations

Patient's plasma dilutions and standard dilutions should yield parallel lines. Absence of parallel lines may indicate an inhibitor in the patient's plasma.

Performance characteristics

Studies have shown that this product will perform as described prior to its expiration date when procedural and storage directions are followed.

Storage and stability

The unopened freeze dried vials are **best stored deep frozen**, but may be stored for up to 3 years at 2 - 8°C without deterioration. Once reconstituted the contents of the vial are then stable for up to 4 hours when held at 2 - 8°C.

Packaging

10 x 1.0 mL or 10 x 2.0 mL (Factor V Depleted Plasma only)

Key guide to symbols

REF	Manufacturers catalogue number.		Consult instructions for use.
LOT	Manufacturers batch number.	Recon.	Requires reconstitution.
IVD	For *in vitro* diagnostic use only.		Product expiry date.
	Biological risks.	+4°C -20°C	Store at 4°C or below. Best stored deep frozen.

Manufacturer.

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Diagnostic Reagents Limited is a BS EN ISO13485:2016 certified company

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